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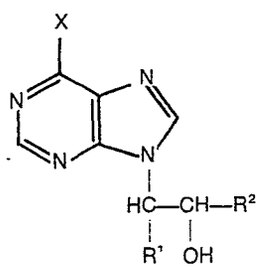
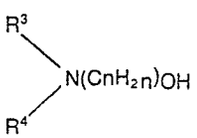
(54) Complexes of 9-hydroxyalkyl-purines, processes for preparing them and therapeutical compositions containing the complexes as active ingredients.

(57) Compounds of the formula



$(Y)_z$

where X is OH, NH_2, SH, OR or SR (where R is alkyl of 1 to 4 carbon atoms or benzyl), R^1 is H or alkyl of 1 to 8 carbon atoms, R^2 is H or methyl, Y is the salt of an amine of the formula



where R^3 and R^4 are lower alkyl, e.g., 1 to 4 carbon atoms and n is an integer of 2 to 4 with p-acetamidobenzoic acid and where z is a number from 0 to 10 are useful as immunomodulators, as antiviral agents and in specific cases have antitumor activity. The compounds and compositions where z is 1 to 10 are novel per se. When R^2 is H the presence of Y enhances the immunoregulatory activity and the antiviral activity. If X is the NH_2 there is immunoinhibitory activity but no immunostimulatory (immunopotentiatory) activity.

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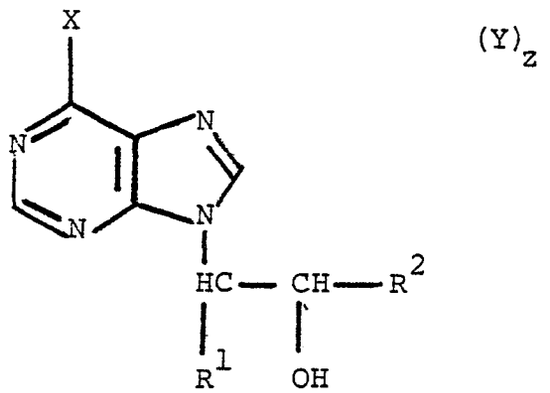
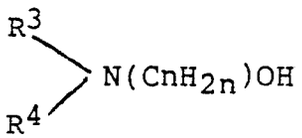
IMMUNOMODULATORS AND ANTIVIRAL AGENTS

SUMMARY OF THE INVENTION

The present invention is based on the discovery that compounds of the formula



where X is OH, NH_2, SH, OR or SR where R is alkyl of 1 to 4 carbon atoms or benzyl, R^1 is H or alkyl of 1 to 8 carbon atoms, R^2 is H or methyl, Y is the salt of an amine of the formula



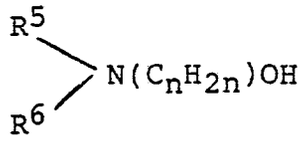
where R^3 and R^4 are lower alkyl, e.g., 1 to 4 carbon atoms and n is an integer of 2 to 4 with p-acetamidobenzoic acid and where z is a number from 0 to 10

are useful as immunomodulators, as antiviral agents and in specific cases have antitumor activity. The compounds and compositions where z is 1 to 10 are novel per se.

When R^2 is H the presence of Y enhances the immunoregulatory activity and the antiviral activity. If X is the NH_2 there is immunoinhibitory activity but no immunostimulatory (immunopotentiatory) activity.

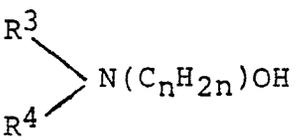
Immunoregulatory activity appears to increase with increasing chain length for R^1, at least from methyl through hexyl. Preferably R^1 is n-alkyl, i.e., methyl, ethyl, n-propyl, n-butyl, n-amyl, n-hexyl, n-heptyl or n-octyl. R^2 is preferably methyl. R can be methyl, ethyl, n-propyl, n-butyl, isopropyl, etc. When X is NH_2 the compound can be present as the free base or as the salt with a non-toxic acid, i.e., pharmaceutically acceptable acid, e.g., hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, citric acid, lactic acids, tartaric acid, salicylic acid, acetyl salicylic acid, acetic acid, propionic acid, p-toluene sulfonic acid, methane sulfonic acid, maleic acid, succinic acid, malonic acid, adipic acid.

A preferred class of amines to form the salt with para acetamidobenzoic acid has the formula



where R^5 and R^6 are lower alkyl, e.g., methyl, ethyl, propyl, isopropyl or butyl and n is an integer of 2 to 4. Typical examples of such amines include dimethylamino ethanol, dimethylamino isopropanol, diethylamino ethanol, diethylamino isobutanol, diethylamino isopropanol, methyl ethyl amino ethanol, diisobutylamino-N-butanol, dimethylamino propanol, dimethylamino-N-butanol, diisobutylamino ethanol, dimethylamino butanol, dibutylamino-N-butanol, dibutylamino ethanol, dipropylamino ethanol and diisopropylamino ethanol. The presently preferred amine is dimethylamino isopropanol. When Y is present, i.e., z is 1 to 10, preferably z is 3. However, z can also be 1, 2, 4, 5, 6, 7, 8, 9 or 10.

While there are preferably used the compounds

where Y is the salt of the amine 

with p-acetamidobenzoic acid there can also be used salts of the formula Y^1 wherein the amine is as just defined the acid is a pharmaceutically acceptable acid other than p-acetamidobenzoic acid, e.g., hydrochloric acid, sulfuric acid, hydrobromic acid, phosphoric acid, acetic acid, propionic acid, malonic acid, lactic acid, citric acid, tartaric acid, p-toluene sulfonic acid, adipic acid, maleic acid, succinic acid, methane sulfonic acid, salicylic acid, acetyl salicylic acid.

In describing the compounds below, when Y is present the abbreviation DIP·PAcBA stands for dimethylamino-2-propanol-p-acetamido benzoate. Unless a number in parentheses, e.g., (10), follows this abbreviation, then Y is 3. If a number in parentheses follows the abbreviation DIP·PAcBA there the number indicates the number of moles of Y groups present to 1 mole of the 9-(hydroxyalkyl)purine.

In Table 1 below the compounds are believed to be pure except for compound 15443 which is believed to also contain a salt in addition to the compound of the invention.

An immunomodulator is a compound which regulates the immune response. Thus, it covers both immunostimulation (immunopotentiation) and immunoinhibition. Immunostimulation, of course, is useful in building up immunity. Immunoinhibition also has utility in a number of areas. For example, it is useful in organ transplants, e.g., kidney or heart transplants, to prevent rejection.

In the tables showing the immunopotentiating properties of the compounds, a plus (+) or a minus (-) indicates immunostimulating or immunoinhibiting properties respectively. The number 0 indicates the compound had neither immunopotentiating activity of immunoinhibiting activity.

There are included in some of the tables several compounds wherein X is not within that claimed. These non-claimed compounds as a rule have relatively low activities and are included to illustrate the fact that the X group can have a significant effect on the properties of the compounds.

A mitogen is a substance which induces cell proliferation, as occurs during immunization.

Table 1 (excluding compounds 15427 and 15423) shows compounds useful in the invention.

The synthetic procedures A through L mentioned in Table 1 are described in more detail subsequently.

The compositions of the invention are useful in treating mammals (and cells of mammals) including humans, swine, dogs, cats, cattle, horses, sheep, goats, mice, rabbits, rats, guinea pigs, hamsters, monkeys, etc.

Unless otherwise indicated, all parts and percentages are by weight.

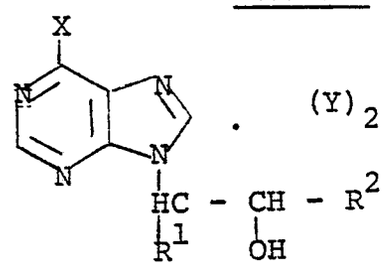
All temperatures are in degrees centigrade unless otherwise indicated.

The compositions can comprise, consist essentially of or consist of the materials set forth and the processes can comprise, consist essentially of or consist of the steps set forth with such materials.

The compositions can be administered to the mammals by conventional techniques, e.g., orally, nasally, rectally, vaginally, enterally or parenterally. They can be employed as injectable solutions, e.g., in water, or as tablets, pills, capsules, etc.

Table 1

SUMMARY OF CHEMICAL PROPERTIES OF 9-(HYDROXYLALKYL PURINES



No.	Compound				Synthetic Method
	R^1	R^2	X	Y	
15425	H	H	OH	-	D
15428	H	H	OH	DIP·PAcBA	L
15435	H	H	SH	-	C
15437	H	H	SH	DIP·PAcBA	L
15446	H	CH_3	OH	-	A
15447	H	CH_3	OH	DIP·PAcBA	L
15431	H	CH_3	NH_2	-	B
15432	H	CH_3	NH_2	DIP·PAcBA	L
15427	CH_3	H	I	-	E
15423	CH_3	H	Cl	-	F
15433	CH_3	H	NH_2	-	G
15434	CH_3	H	NH_2	DIP·PAcBA	L
15443	CH_3	H	OH	-	H
15444	CH_3	H	OH	DIP·PAcBA	L
15417	C_6H_{13}	H	OH	-	I
15418	C_6H_{13}	H	OH	DIP·PAcBA	L
15392	C_6H_{13}	CH_3	OH	-	J
15410	C_6H_{13}	CH_3	OH	DIP·PAcBA	L
15426	C_6H_{13}	CH_3	NH_2	HCl Salt	K

No.		UV Spectra				Elemental Analysis			
	M.Pt. °C	λMax	λMin	Con 10^{-3}	pH	C	H	N	
		250	222.5	11.93	7				
15425	274°	250	219	11.0	1				
		254	221.5	12.53	10				
15428									
		323	251	23.0	7				
15435	278-80	323	252	19.9	1				
		323	251	19.9	10				
15437									
		250	223.5	11.0	7				
15446	244-5	250	220	10.6	1				
		254	223.5	12.1	10				
15447									
		261	228	15.8	7	Cal	49.73	5.74	36.25
15431	188°	259	231	15.4	1	FD	49.56	5.62	36.22
		261	225	15.7	10				
15432									
		276	237	10.9	7	Cal	31.60	2.98	18.43
15427	178°	276	237	10.9	1	FD	31.53	2.96	18.18
		276	237	10.9	10				
		265	228	9.1	7	Cal	45.20	4.26	26.36
15423	200-204	265	228	9.1	1	FD	45.11	4.27	26.25
		265	228	9.1	10				
		261.5	228	13.56	7				
15433	215-16	259	231	13.26	1				
		261	224.5	13.80	10				
15434									
		250	223	7.52	7				
15443	198-199	250	218	6.91	1				
		255	225.5	7.91	10				
15444									
		250	224	11.09	7	Cal	59.07	7.65	21.16
15417	226°C	250	220	10.37	1	FD	59.01	7.55	21.24
		255	223	11.96	10				
15418									
		250	224	12.1	7	Cal	60.41	7.97	20.13
15392	202 °C	248	222	13.3	1	FD	60.47	7.86	20.08
		254	220	14.1	10				
15410									
		261	230	9.77	7	Cal	53.58	7.71	22.32
15426	176-9 °C	259	233	9.60	1	FD	53.56	7.67	22.34
		251	235	9.77	10				

Other compounds within the invention are set forth in Table 1a below wherein the basic formula is the same as that in Table 1. In Tables 1 and 1a, the alkyl groups for R^1 are all n-alkyl.

Table 1a

COMPOUND

R^1	R^2	X	Y
C_6H_{13}	CH_3	OH	DIP·PAcBA(10)
C_6H_{13}	CH_3	OH	DIP·PAcBA(1)
H	CH_3	OH	DIP·PAcBA(10)
H	CH_3	OH	DIP·PAcBA(1)
CH_3	CH_3	OH	-
CH_3	CH_3	OH	DIP·PAcBA
C_2H_5	H	OH	DIP·PAcBA
C_2H_5	H	OH	-
C_3H_7	H	OH	-
C_3H_7	H	OH	DIP:PAcBA
C_2H_5	CH_3	OH	-
C_2H_5	CH_3	OH	DIP·PAcBA
C_2H_7	CH_3	OH	-
C_3H_7	CH_3	OH	DIP·PAcBA
C_4H_9	H	OH	-
C_4H_9	H	OH	DIP·PAcBA
C_4H_9	CH_3	OH	-
C_4H_9	CH_3	OH	DIP·PAcBA

Table 1a (cont.)

COMPOUND				
	R^1	R^2	X	Y
	C_5H_{11}	H	OH	-
	C_5H_{11}	H	OH	DIP·PAcBA
	C_5H_{11}	CH_3	OH	DIP·PAcBA
	C_5H_{11}	CH_3	OH	-
	C_7H_{15}	H	OH	-
	C_7H_{15}	H	OH	DIP·PAcBA
	C_7H_{15}	CH_3	OH	-
	C_7H_{15}	CH_3	OH	DIP·PAcBA
	C_8H_{17}	H	OH	-
	C_8H_{17}	H	OH	DIP·PAcBA
	C_8H_{17}	CH_3	OH	-
	C_8H_{17}	CH_3	OH	DIP·PAcBA
	C_6H_{13}	CH_3	OCH_3	-
	C_6H_{13}	CH_3	OCH_3	DIP·PAcBA
	C_6H_{13}	H	OCH_3	DIP·PAcBA
	C_6H_{13}	H	OCH_3	-
	CH_3	H	OCH_3	-
	CH_3	H	OCH_3	DIP·PAcBA
	H	H	OCH_3	-
	H	H	OCH_3	DIP·PAcBA
	H	CH_3	OCH_3	DIP·PAcBA
	H	CH_3	OCH_3	-

Table 1a (cont.)

COMPOUND

	R^1	R^2	X	Y
	C_6H_{13}	CH_3	OC_2H_5	-
	C_6H_{13}	CH_3	OC_2H_5	DIP·PAcBA
	C_6H_{13}	H	OC_2H_5	DIP·PAcBA
	C_6H_{13}	H	OC_2H_5	-
	C_6H_{13}	CH_3	OC_3H_7	-
	C_6H_{13}	CH_3	OC_3H_7	DIP·PAcBA
	CH_3	H	OC_3H_7	DIP·PAcBA
	CH_3	H	OC_3H_7	-
	H	H	OC_3H_7	DIP·PAcBA
	H	CH_3	OC_3H_7	DIP·PAcBA
	C_6H_{13}	CH_3	OC_4H_9	-
	C_6H_{13}	CH_3	OC_4H_9	DIP·PAcBA
	C_6H_{13}	H	OC_4H_9	-
	C_6H_{13}	H	OC_4H_9	DIP·PAcBA
	H	H	OC_4H_9	-
	H	H	OC_4H_9	DIP·PAcBA
	H	CH_3	OC_4H_9	-
	H	CH_3	OC_4H_9	DIP·PAcBA
	CH_3	CH_3	OC_4H_9	-
	CH_3	CH_3	OC_4H_9	DIP·PAcBA
	CH_3	H	OC_4H_9	-
	CH_3	H	OC_4H_9	DIP·PAcBA

Table 1a (cont.)

COMPOUND			
R^1	R^2	X	Y
C_6H_{13}	CH_3	SCH_3	-
C_6H_{13}	CH_3	SCH_3	DIP·PAcBA
C_6H_{13}	H	SCH_3	-
C_6H_{13}	H	SCH_3	DIP·PAcBA
CH_3	CH_3	SCH_3	-
CH_3	CH_3	SCH_3	DIP·PAcBA
CH_3	H	SCH_3	-
CH_3	H	SCH_3	DIP·PAcBA
H	H	SCH_3	-
H	H	SCH_3	DIP·PAcBA
H	CH_3	SCH_3	DIP·PAcBA
H	CH_3	SCH_3	-
C_6H_{13}	CH_3	SC_4H_9	-
C_6H_{13}	CH_3	SC_4H_9	DIP·PAcBA
C_6H_{13}	H	SC_4H_9	DIP·PAcBA
C_6H_{13}	H	SC_4H_9	-
CH_3	H	SC_4H_9	-
CH_3	H	SC_4H_9	DIP·PAcBA
H	H	SC_4H_9	-
H	H	SC_4H_9	DIP·PAcBA
H	CH_3	SC_4H_9	DIP·PAcBA
H	CH_3	OH	DIP·PAcBA(10)
H	CH_3	OH	DIP·PAcBA(1)

Table 1a (cont.)

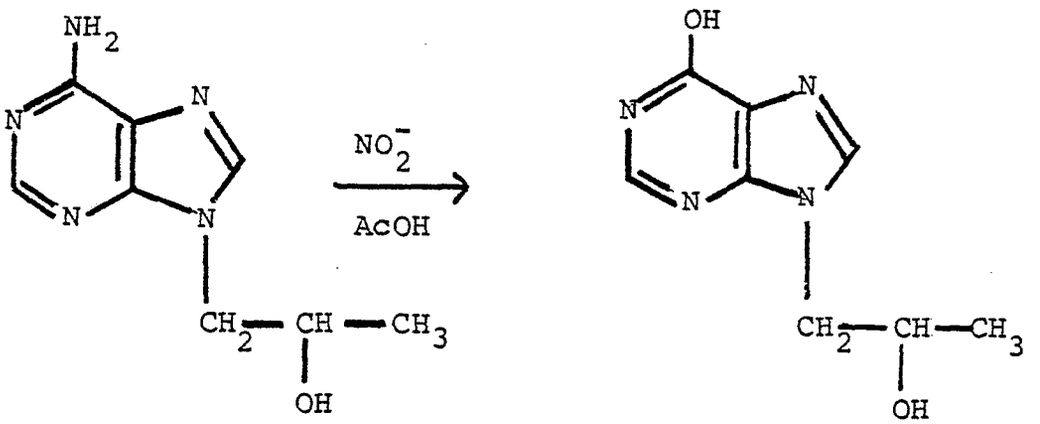
COMPOUND

	R^1	R^2	X	Y
	C_6H_{13}	H	O-benzyl	-
	C_6H_{13}	H	O-benzyl	DIP·PAcBA
	C_6H_{13}	CH_3	O-benzyl	-
	C_6H_{13}	CH_3	O-benzyl	DIP·PAcBA
	C_6H_{13}	CH_3	S-benzyl	-
	C_6H_{13}	CH_3	S-benzyl	DIP·PAcBA
	C_6H_{13}	H	S-benzyl	-
	C_6H_{13}	H	S-benzyl	DIP·PAcBA

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Method A

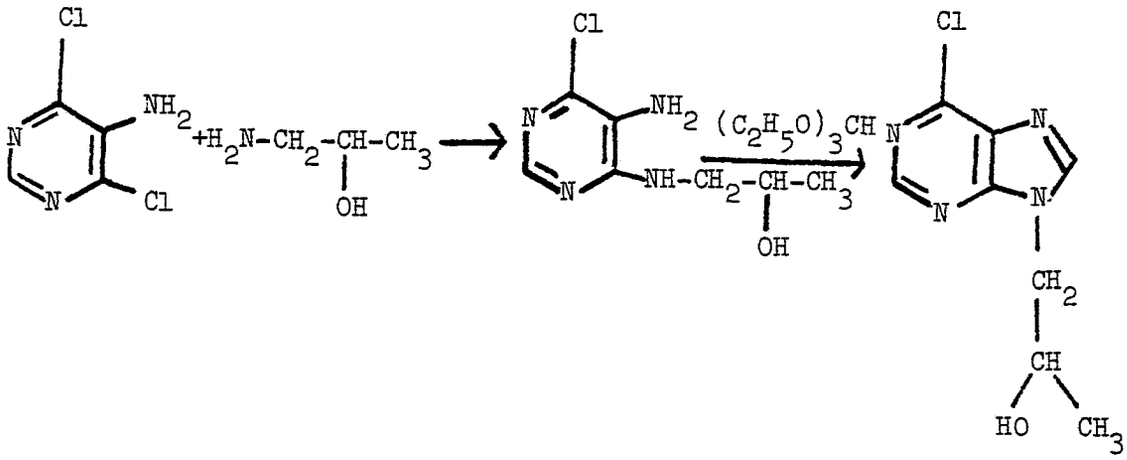
9-(2-HYDROXY-1-PROPYL)HYPOXANTHINE (NPT 15446)



9-(2-Hydroxy-1-propyl)adenine (I, 4.0 g, 20.7 mmol) was suspended in 50% acetic acid (20 ml) and sodium nitrite (4 g, 58 mmol), was slowly added. The mixture was stirred at 25° for 3 hr. The resulting solution was evaporated to dryness and isopropanol added; this operation was repeated once. The solid residue was boiled in isopropanol and filtered. The filtrate was evaporated and crystallized by addition of acetone. Recrystallization was made from iso-propanol/methanol (98:2); a colorless crystalline product was obtained. Yield 3.3 g (82%) M.P. 244-250°
uv (H_2O; pH 5.5) λ max 250 nm.

Method B

9-(2-HYDROXY-1-PROPYL)-6-CHLOROPURINE



There were employed the methods of Schaeffer, H.J. Vogel, D. and Vince, R., J. Med. Chem. 8,502 (1965); and Schaeffer, H.J. and Vince, R., J. Med. Chem. 10, 689 (1967).

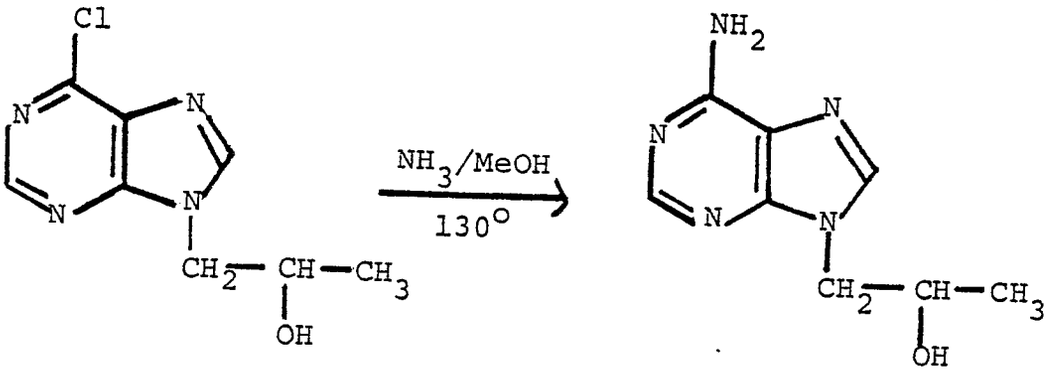
A solution of 5-amino-4,6-dichloropyrimidine (I, 20 g, 0.12 mole) in 11% ethanolic solution of isopropanolamine (200 ml) was refluxed for 8 hr. The reaction mixture was evaporated to a syrup, ethanol added and evaporated again; this operation was repeated once. The resulting syrup was poured into water (300 ml) giving a crystalline mass. It was collected by filtration, washed with water and dried to give 19 g of crude 9-(2-hydroxy-1-propylamino) 5-amino-6 chloropyrimidine (II).

The crude compound II was suspended in triethylorthoformate (120 ml) to which ethanesulfonic acid (5 drops) was added. After 15 min. all the solid

dissolved and the solution was kept a 25^0 overnight. Evaporation in vacuo gave a thick syrup which was submitted to high vacuo evaporation to remove the excess of isopropanolamine. Upon crystallization with xylene, 5 g of crude material was obtained.

Method B

9-(2-HYDROXY-1-PROPYL)ADENINE (NPT 15431)

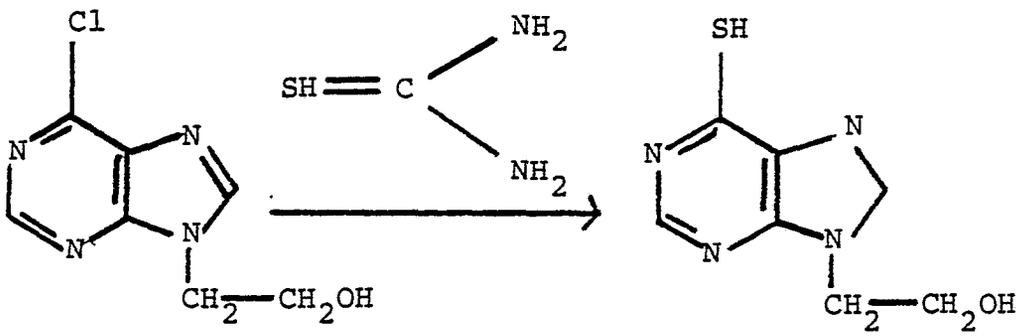


9-(2-Hydroxy-1-propyl)-6chloropyrine (I,9 g, 42.4 (mol) was dissolved in saturated methanolic ammonia and ammonium chloride (50 mg). The mixture was heated at 130° in a bomb for 6 hr. The resulting solution was evaporated to dryness and recrystallized from ethanol/acetone. Yield = 6.68 g of a colorless crystalline product (81%) mp 193-194° uv (H_2O; pH 5.5) λmax 260 nm TLC in $CHCl_3$:MeOH (5:1) R_f 0.44

Anal. Calc. for $C_8H_{11}N_5O$: C, 49.73; H, 5.74; N, 36.25; Found: C, 49.56, H, 5.62; N, 36.22.

Method C

9-(1-HYDROXYETHYL)-6-MERCAPTOPURINE (NPT 15435)



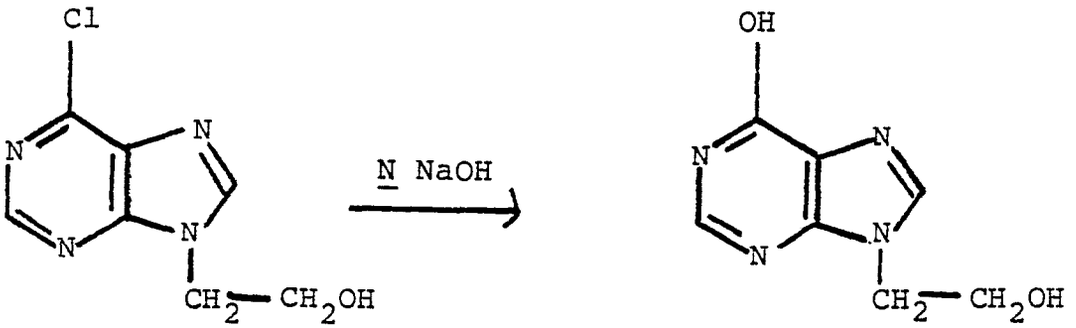
There was employed the method of Schaeffer and Bhargava, Biochemistry 4, 71 (1965).

9-(1-Hydroxyethyl)-6-chloropurine (I, 2 g, .01 mol) and thiourea (0.76 g; .01 mol) were dissolved in ethanol (15 ml) and refluxed for 30 min. The resulting precipitate was collected by filtration and suspended in water to form a slurry. Neutralization with sodium acetate gave colorless crystals. Yield 1.5 g (76%).

M.P. 278-280°; uv (H_2O, pH 5.5) λmax 320, 230 nm.

Method D

9-HYDROXYETHYL HYPOXANTHINE (NPT 15425)

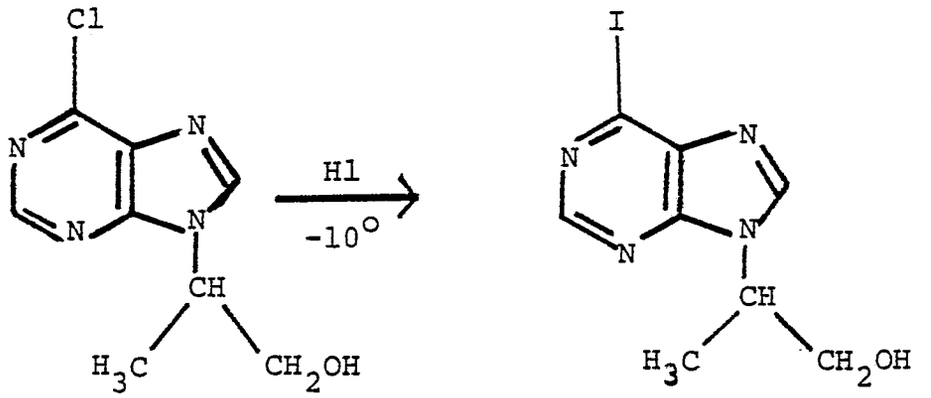


There was used the method of Schaeffer, H.J. and Bhargava, P.S., Biochemistry 4, 71 (1965).

6-Chloro-9-hydroxyethyl purine, III (4 g), was added slowly to warm N NaOH (30 ml) and refluxed for 2 hr. The reaction is cooled in ice and neutralized with glacial acetic acid. After filtration, portions of unreacted III are removed. The product is recrystallized from methanol and washed with acetone. Colorless crystals. Yield, 1 g. (28%); mp 274°; uv (H_2O, pH 5.5), λ max 250 nm.

Method E

9-(1-HYDROXYL-2-PROPYL)-6-IODOPURINE (NPT 15427)

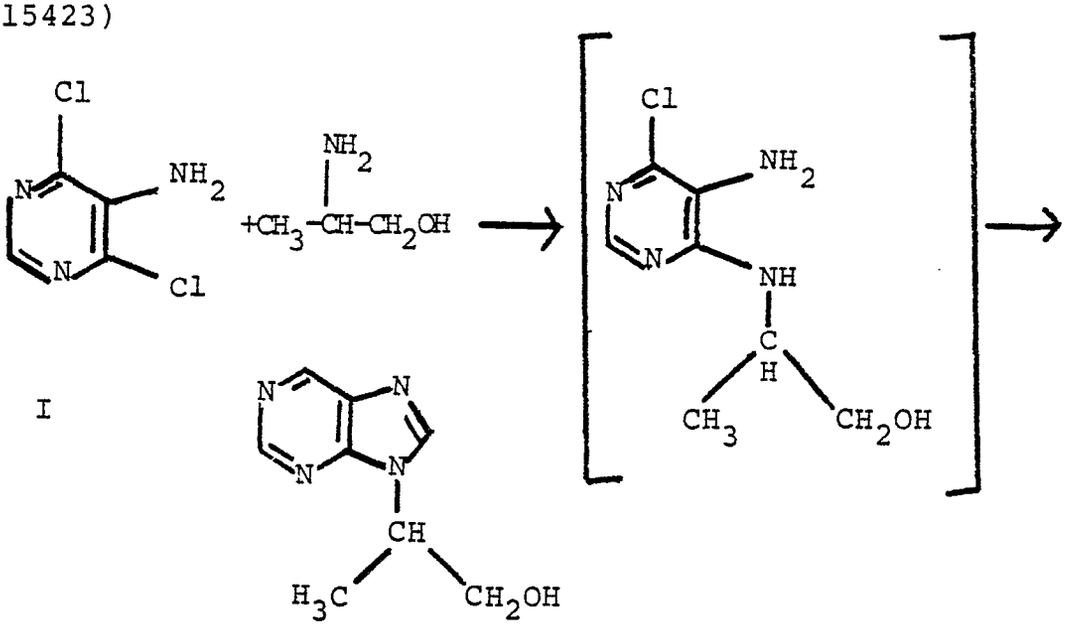


9-(1-Hydroxy-2-propyl)-6-chloropurine (I, 1.5 g, 7 mmol) was added to hydroiodic acid (15 ml) at -10° with stirring for 45 min. The precipitate was filtered, neutralized with anhydrous sodium acetate at 5°, and washed with a little cold water (3 times). Recrystallization from ethanol/H_2O, gave colorless crystals. Yield = 0.9 g (42%) mp = 193-194° uv λmax 276 nm (H_2O, pH 5.5).

Anal. Calc. for $C_8H_9N_4OI$ MW = 304.1: C, 31.60; H, 2.98; N, 18.43; I, 41.73. Found: C, 31.53; H, 2.96; N, 18.18; I, 41.70.

Method F

9-(1-HYDROXY-2-PROPANE)-6-CHLOROPURINE (NPT 15423)



I

There was used the method of Schaeffer, H.J. and Schwender, C.F., J. Med. Chem. 17, 6 (1974).

A solution of 5-amino-4,6-dichloropyrimidine (I, 6.56 g 40 mmol) and 2-amino-1-propanol (II, 3.3 g, 44 mmol) was refluxed in n-pentanol (288 ml) and tert-butylamine (96 ml) for 45 hr. under N_2 atmosphere. The solution was evaporated to a syrup and ethanol added 4 times and evaporated. The resulting syrup was suspended in triethylorthoformate (150 ml) and ethanesulfonic acid (10 drops). The suspension was vigorously stirred overnight, then evaporated to dryness, ethanol added and this operation repeated three times. Crystallization of colorless product occurs during

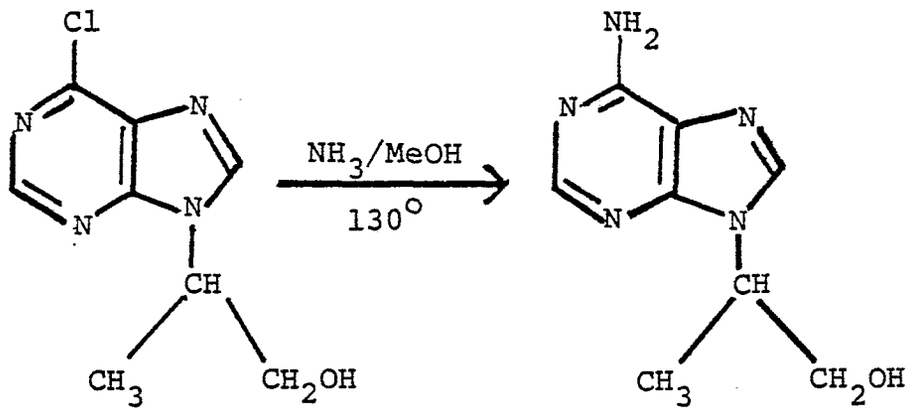
evaporation. The crystals were filtered, and the filtrate was evaporated, ethanol added and this operation repeated three times to give a crude material (3.6 g).

Recrystallized from 98% aqueous ethanol. uv (H_2O, pH 5.5) λmax 265 nm; mp 201-203°; yield 2.79 (32%).

Anal. $C_8H_9N_4OCl$. Calc. C, 45.20; H, 4.26; N, 26.36; Cl, 16.68. Found: C, 45.11; H, 4.27; N, 26.25; Cl, 16.71.

Method G

9-(1-HYDROXY-2-PROPYL)ADENINE (NPT 15433)

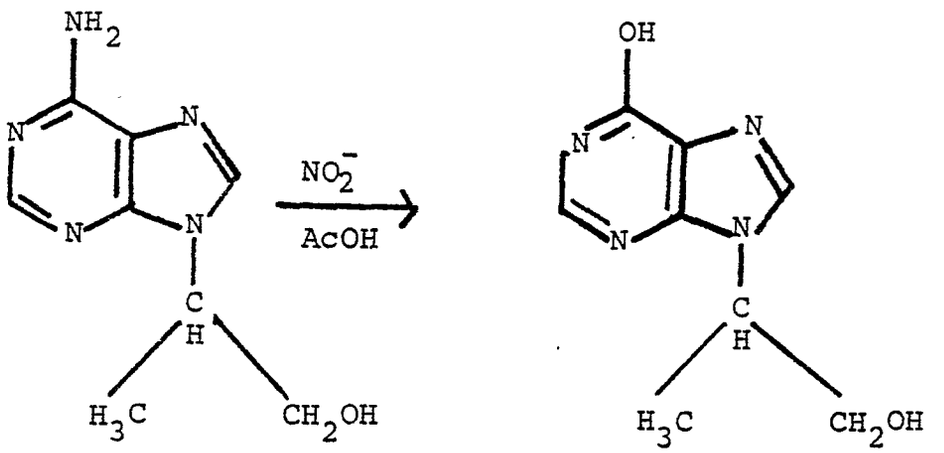


There was used the procedure of Schaeffer, H. and Schwender, C., J. Pharm. Sci., 60, 1204 (1971). Also Schaeffer et al., J. Med. Chem. 15, 456 (1972).

9-(1-Hydroxy-2-propyl)-6-chloropurine (I, 2.0 g, 9.4 mmol) was suspended in methanol/ammonia (30 ml) and ammonium chloride (50 mg) added as a catalyst and the mixture heated at 130° for 4.5 hr.; the solution was evaporated to dryness. Recrystallization from ethanol of the obtained crude product gave colorless needles. Yield = 1.15 g (63%) mp = 215-216° uv (H_2O, pH 5.5) λmax 260 nm.

Method H

9-(1-HYDROXY-2-PROPYL)HYPOXANTHINE (NPT 15443)



I II

9-(1-Hydroxy-2-propyl)adenine (I, 4 g, 21 mmol) was dissolved in 50% acetic acid (20 ml), sodium nitrite (4 g, 58 mmol) added and the mixture stirred at 25° for 3-1/2 hr. The solution was evaporated to dryness twice with isopropanol. The residue was taken up in isopropanol and filtered, the precipitate discarded, and the filtrate evaporated to form a gel which, upon the addition of acetone, solidified. Yield = 3.65 (90%) of colorless crystals. Recrystallized from isopropanol/methanol (98:2). mp = 202-207° TLC in $CHCl_3$:MeOH (5:1) 1 spot R_f - 0.30 uv (H_2O, pH 5.5) = λmax 250 nm.

Method I

COMPOUND NPT 15417

There was used the procedure of Schaeffer et al, Journal of Pharmaceutical Sciences 16:1204-1210, Method F.

The product is compound XL in Table III of Schaeffer et al.

Method J

ERYTHRO-9-(2-HYDROXY-3-NONYL)HYPOXANTHINE (NPT 15392)

An outline of the synthetic sequence for the preparation of erythro-9-(2-hydroxy-3-nonyl)hypoxanthine (Nonylhypoxanthine, VIII) is shown in Flow Charts 1 and 2. The improvements over the procedure

of H.J. Schaeffer and C.F. Schwender, J. Med. Chem., 17, 6 (1974) in the reaction sequence leading to the erythro-9-(2-hydroxy-3-nonyl)-6-chloropurine (VII) are indicated. The last step, the hydrolysis of the 6-chloropurine derivative (VII), to yield nonylhypoxanthine (VIII) is an adaptation of the method reported by A. Giner-Sorolla, C. Gryte, A. Bendich and G.B. Brown, J. Org . Chem. 34, 2157 (1969) for the hydrolysis of halogenopurines.

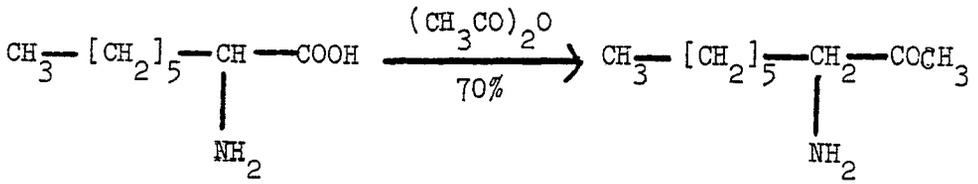
The alternate route, i.e., the nitrosation of erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) (IX), to yield Nonylhypoxanthine (VIII) (shown on Flow Chart 2) consists of the previous conversion by ammonolysis of the chloro derivative (VII) into the aminopurine (IX, EHNA) followed by its nitrosation to yield Nonylhypoxanthine (VIII).

Flow Chart 1

OUTLINE OF THE SYNTHESIS OF
ERYTHRO-9-(2-HYDROXY-3-NONYL)HYPOXANTHINE (VIII)

Step 1 ACETAMIDONONAN-2-ONE (II)

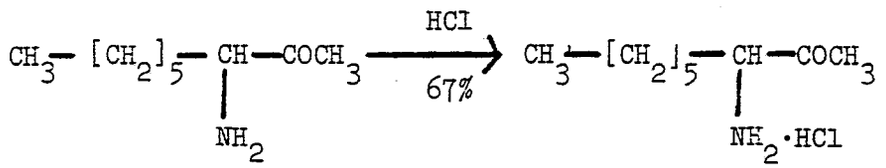
Acylation of 2-amino octanoic acid



I II

Step 2 ACETAMIDONONAN-2-ONE HYDROCHLORIDE (III)

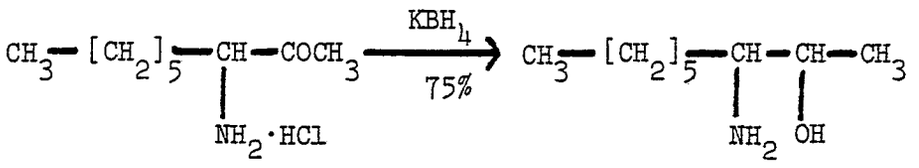
Formation of the acetamidononan-2-one hydrochloride



II III

Step 3 ERYTHRO-3-AMINO-2-NONANOL (IV)

Reduction of the acetamidononan-2-one hydrochloride

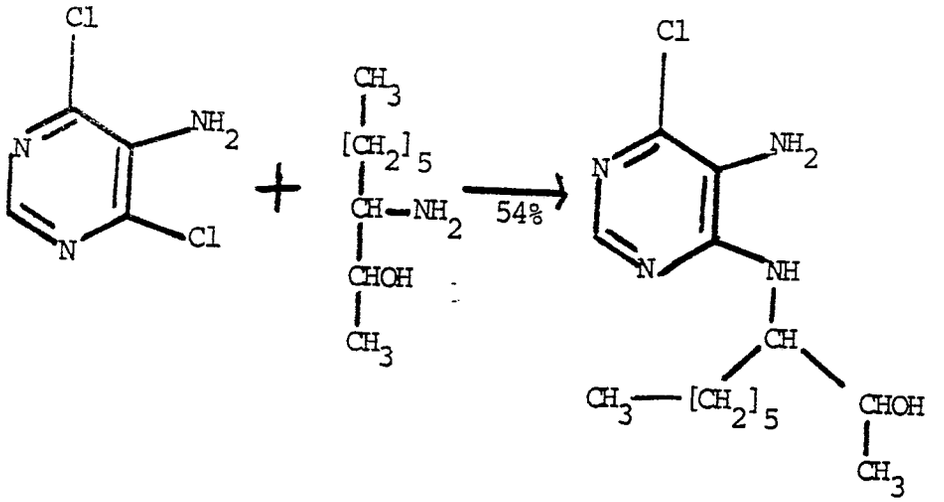


III IV

(Figures below the arrow refer to % yield.)

Step 4 ERYTHRO-5-AMINO-4-CHLORO-6-(2-HYDROXY-3-NONYLAMINO)PYRIMIDINE (VI)

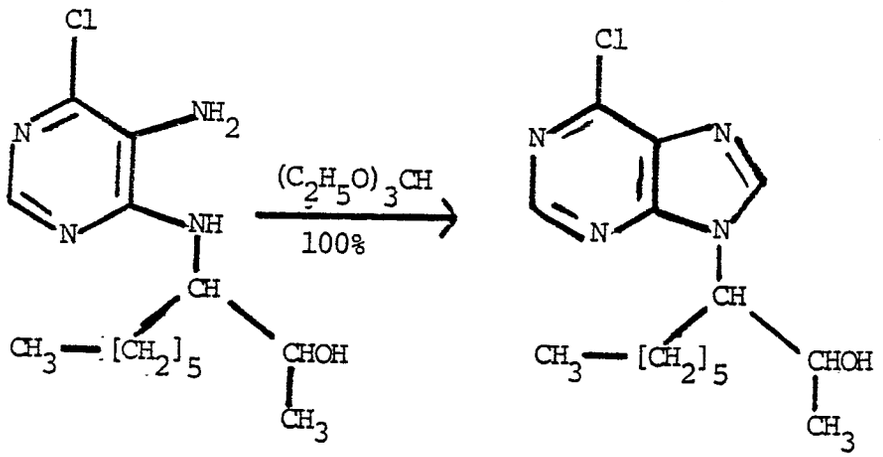
Condensation of erythro-3-amino-2-nonanol with 5-amino-4,6-dichloropyrimidine



V IV VI

Step 5 ERYTHRO-9-(2-HYDROXY-3-NONYL)-6-CHLOROPURINE (VII)

Ring closure of erythro-5-amino-4-chloro-6-(2-hydroxy-3-nonylamino)pyrimidine (V)

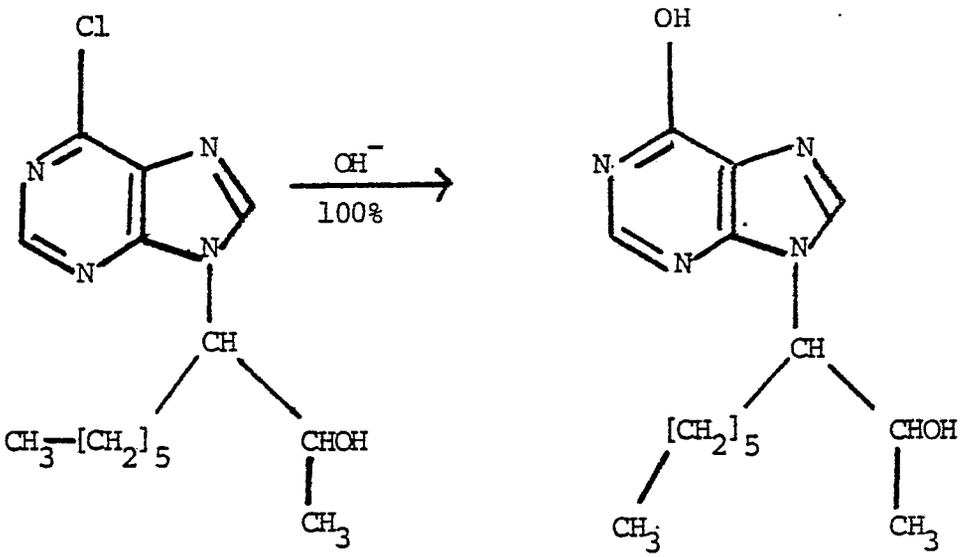


VI VII

Flow Chart 1 (cont.)

Step 6 ERYTHRO-9-(2-HYDROXY-3-NONYL)HYPOXANTHINE (VIII)

(By hydrolysis of the 6-chloropurine derivative)



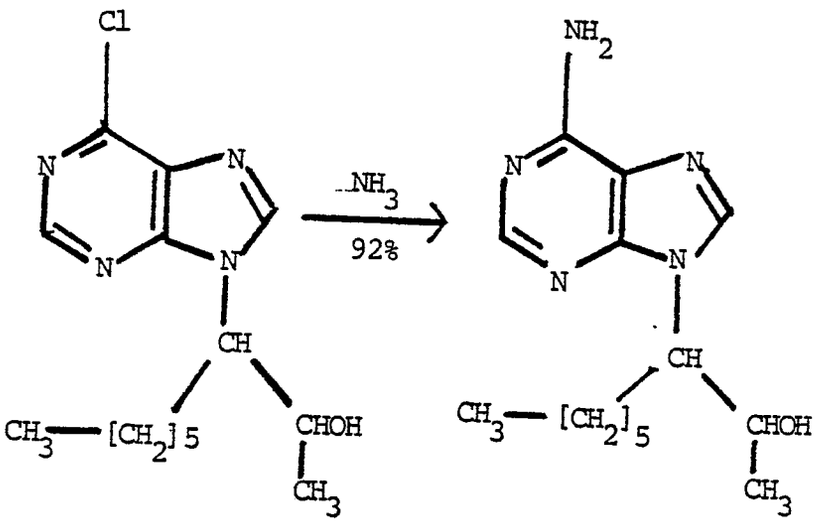
VII VIII

Flow Chart 2

ALTERNATIVE ROUTE FOR THE PREPARATION OF ERYTHRO-9-(2-HYDROXY-3-NONYL HYPOXANTHINE (VIII)

Step 1a ERYTHRO-9-(2-HYDROXY-3-NONYL)ADENINE (IX)

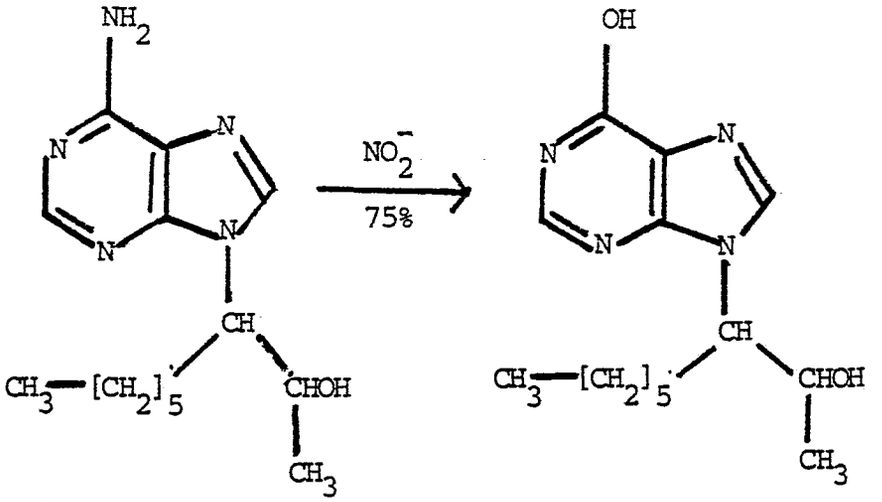
Ammonolysis of erythro-9-(2-hydroxy-3-nonyl)-6-chloropurine (VII)



VII IX

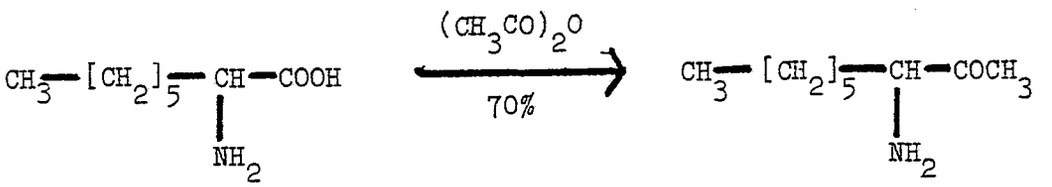
Step 2b ERYTHRO-9-(2-HYDROXY-3-NONYL)HYPOXANTHINE (VIII)

Nitrosation of erythro-9-(2-hydroxy-3-nonyl)adenine (IX)



IX VIII

3-ACETAMIDONONAN-2-ONE (II)

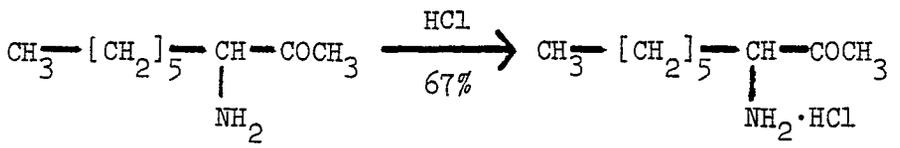


I II

A mixture of 2-amino-1-octanoic acid (I, 200 g, 1.26 mole) in acetic anhydride (960 ml), and pyridine (640 ml) was heated on a boiling water bath for 4 hr. The reaction mixture was evaporated in vacuo, and the residue was partitioned 6-8 times between 5% aqueous solution of $NaHCO_3$ (400 ml) and ether (400 ml).

The combined ethereal extracts were dried with anhydrous $MgSO_4$ and evaporated to dryness to give crude 3-acetamidononan-2-one, 154 g (70%).

3-AMINO-2-NONANONE HYDROCHLORIDE (III)

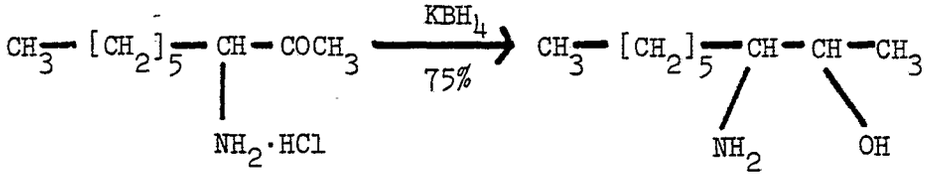


II III

The crude product (II) obtained in the preceding operation (154 g) was dissolved in concentrated aqueous HCl (1,540 ml) and refluxed for 2 hr. and then evaporated to dryness in vacuo. The resulting solid was recrystallized from a warm solution in EtOH (200 ml) and then cooled to 25°. To this solution ether (600 ml) was added. A white crystalline precipitate appears; the suspension is kept at 5° overnight. The precipitate is collected and washed with ether (once with 100 ml) to give 125 g (67%) white crystalline product M.P. 112° dec.

If the crystalline material were not white or had a lower melting point, it should be recrystallized with charcoal from tetrahydrofuran. In one repeat of this procedure there was used 150 ml of hydrofuran for 100 g of the crude hydrochloride (III).

ERYTHRO-3-AMINO-2-NONANOL (IV)



III IV

3-Amino-2-nonanol hydrochloride (43.8 g, 0.226 mole) was dissolved in absolute methanol (150 ml) and cooled to -10° in an ice-salt bath. 1/ Potassium borohydride (24.4 g, 0.45 mole) 2/ was added in small portions over a 2-3 hr. period. The mixture is then kept at -10° to -15° for 3 hr. 3,4/ and slowly allowed to reach room temperature (22°), then stirred overnight (20 hr.) at room temperature. The mixture is then evaporated to dryness (syrup) in vacuo and partitioned between H_2O (150 ml) and chloroform (150 ml). The H_2O layer was further extracted (3x) with chloroform (100 ml ea.). The chloroform layer was dried with $MgSO_4$ and evaporated in vacuo to give a slightly yellowish, oily product. This liquid was distilled in high vacuo at 95°-100° (0.15 mm Hg) to give pure erythro-3-amino-2-nonanol, 26.4 g, 75% yield, m.p. 81°-86°.

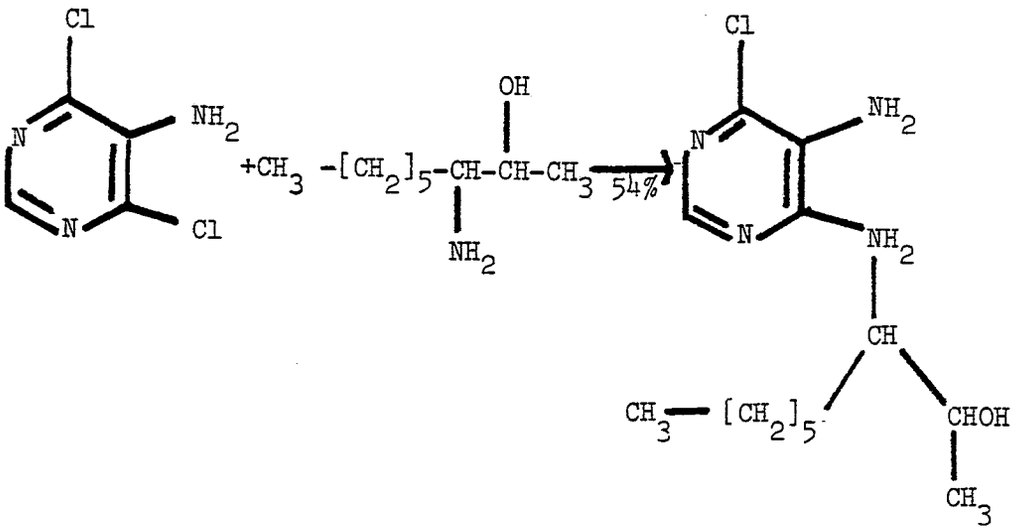
1. Upon cooling the solution of III, some material precipitates; this has no effect on the outcome of the reaction.

2. At this point, the present procedure differs from that of Schaeffer et al. Schaeffer adds acetic acid at the same time as KBH_4, maintaining the pH at 5-6. It has been found that neutralization entails loss of KBH_4 and that a pH above 5 is tolerated. More important is the fact that the simultaneous addition of acetic acid and KBH_4 (as proposed by Schaeffer) makes the reaction very difficult to control. The temperature raises considerably and losses in yield and/or quality of the product occur.

3. It is recommended to use an efficient stirring to insure the proper reaction which will be completed when all the small lumps and portions of potassium borohydride have disappeared.

4. Cooling at 0°, as described by Schaeffer et al (Method D, line 4 and ff.) is insufficient. It is an improvement to keep the reaction well below 0°; it is best to keep it below -10° all the time. If the temperature is allowed to go over -10°, substantial loss in yield may result.

ERYTHRO-5-AMINO-4-CHLORO-6-
(2-HYDROXY-3-NONYLAMINO)PYRIMIDINE (VI)



V IV VI

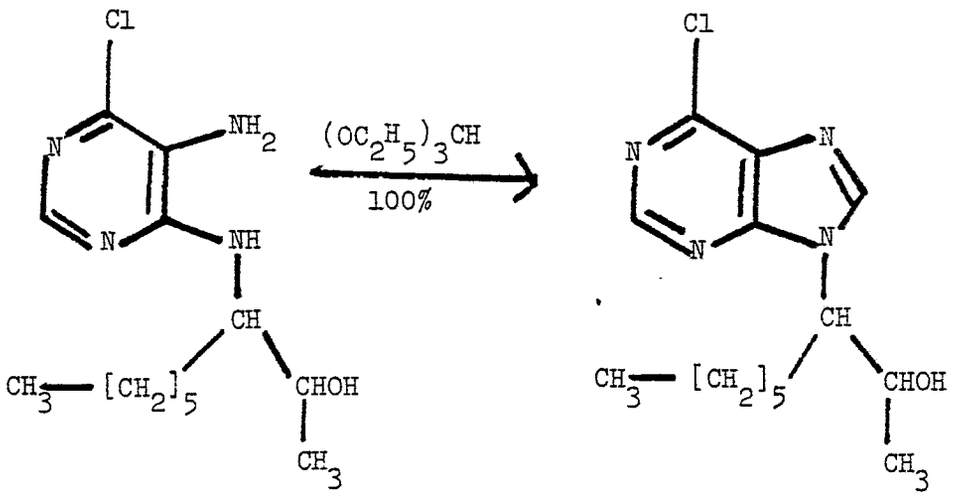
A mixture of 4,6-dichloro-5-aminopyrimidine (V, 24.6, 0.15 mole) and erythro-3-amino-2-nonanol (IV, 26.2 g, 0.164 mole) in 1-pentanol (1.080 ml) and tributylamine (350 ml) was prepared with stirring at 25°. The resulting suspension was heated to reflux under nitrogen atmosphere for 28 hr. (solution took place in about 1/2 hr.). At that time a sample of the reaction product showed a uv λ max 267 and 297 nm (H_2O, pH 5.5).

The resulting solution was concentrated in a hot water bath at 10 mm pressure to a syrup and further evaporated in an oil bath at 0.1 mm and 100° to

yield a viscous liquid to which n-hexane (450 ml) was added. The mixture was refluxed for 1 hr., and the hot, yellowish hexane supernatant was separated from the liquid at the bottom of the round bottom flask.

The resulting light brown oil from which any residual hexane was evaporated in vacuo and dissolved in chloroform (150 ml). This chloroform solution was extracted 8 times with an aqueous saturated solution of $NaHCO_3$ (250 ml each time). The chloroform layer was then separated, dried (with sodium or magnesium sulfate) and evaporated under high vacuo (0.1 mm) at 40° (water bath) to give a light brown oil which solidified on cooling. This material can be used directly in the next step or purified as follows: The resulting oil was dissolved in 75-100 ml chloroform and n-hexane (ca. 300 ml) added to precipitate out a white crystalline solid which was filtered from the cooled solution. (Extraction is carried out 4-8 times, until carbon dioxide is no longer evolved.) This treatment was repeated two more times. Yield: 23.3 g (54%) uv λmax 267, 297 (H_2O, pH 5.5) mp 113-116°.

ERYTHRO-9-(2-HYDROXY-3-NONYL)6-CHLOROPURINE (VII)

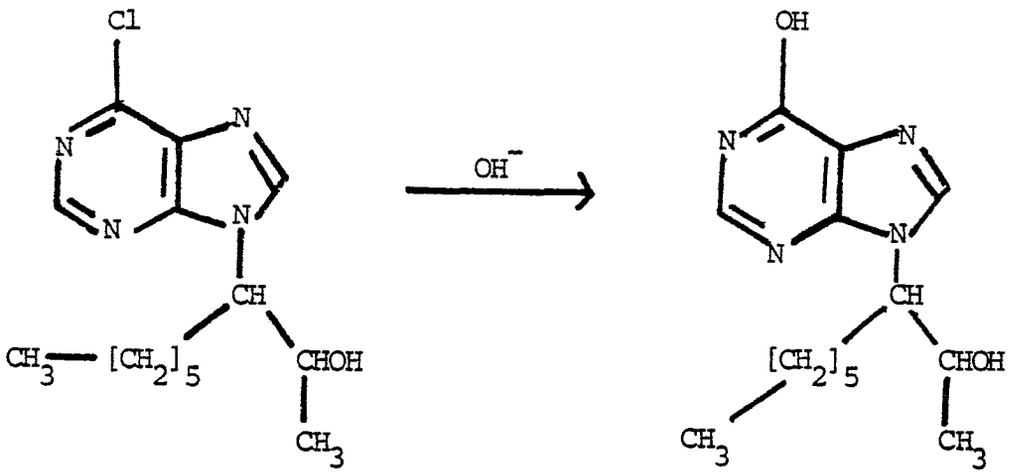


VI VII

The crude syrup from the preceding operation consisting of erythro-5-amino-4-chloro-6-(2-hydroxy-3-nonylamino)pyrimidine (11.48 g, 40 mmol.) was dissolved in triethylorthoformate (106 ml) and chloroform (34 ml), ethanesulfonic acid (10 drops) was added to effect solution. After standing overnight at 25°, the solution was evaporated to a syrup under vacuo. Yield 11.7 g (quantitative). This syrup consisting of crude erythro-9-(2-hydroxy-3-nonyl)-6-chloropurine (VII) was used in the next step. λMax. 264 nm.

ERYTHRO-9-(2-HYDROXY-3-NONYL)HYPOXANTHINE (VIII)

(By hydrolysis of the 6-chloropurine derivative)



VII VIII

A suspension of erythro-6-chloro-9-(2-hydroxy-3-nonyl)purine (VII, 4.0 g, 13.4 mmol) in 0.5 N NaOH (40 ml) was refluxed for 2 hr. and cooled. Neutralization with glacial acetic acid and cooling gave a crystalline precipitate of erythro-9-(2-hydroxy-3-nonyl)hypoxanthine (VIII) which was filtered and dried. Yield: 3.8 g(quantitative), m.p. 196° uv λmax (pH 5.5) 251 nm.

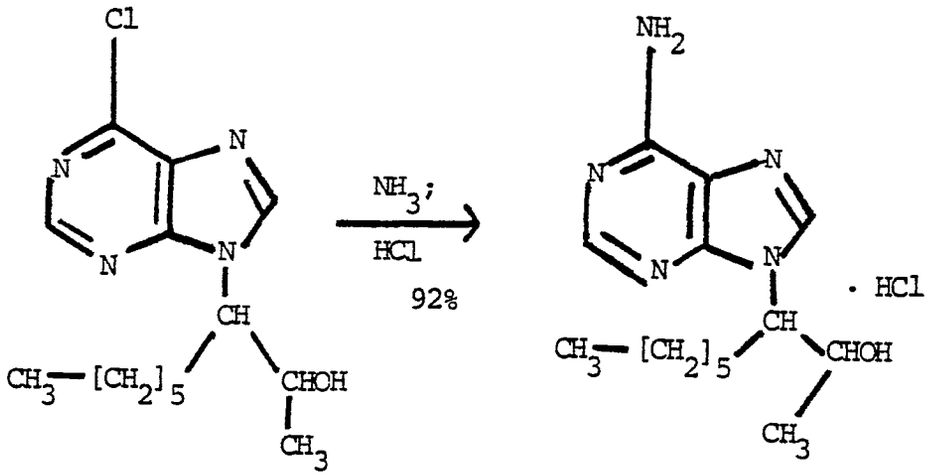
The crude product (VIII) thus obtained was homogeneous by paper chromatography (3 solvents) and gave negative test for Cl^- (copper wire and flame; sodium fusion, acidification and silver nitrate).

Recrystallization of a sample of the crude material 3 times from aqueous ethanol (see Purification) gave colorless crystals. m.p. 202°. Calc. for $C_{14}H_{22}N_4O_2$ (VIII): C, 60.41; H, 7.97, N, 20.13. Found: C, 60.47; H, 7.86; N, 20.08.

PURIFICATION OF
ERYTHRO-9-(2-HYDROXY-3-NONYL)HYPOXANTHINE (VIII)

The crude nonyl hypoxanthine (VIII) is purified by recrystallization. The crude material is dissolved by heating in about 6-10 times its weight in ethyl alcohol, and then an equal volume of H_2O is added. The solution is treated with charcoal in an Erlenmeyer and filtered through celite when hot. The solution is evaporated with continuous stirring on a hot plate. Water is added in small portions to replace the evaporated volume until an abundant precipitate appears. Keep on evaporating the solvent to remove all the ethyl alcohol while adding repeatedly H_2O to reach a volume of 8-12 times the weight of material. The loss in material is about 10% per each recrystallization. Two recrystallizations raised the melting point to 202° and gave a colorless crystalline product while the crude material was somewhat yellow or pink and melted at 192°.

ERYTHRO-9-(2-HYDROXY-3-NONYL)-ADENINE. HCl (IX)



VII IX

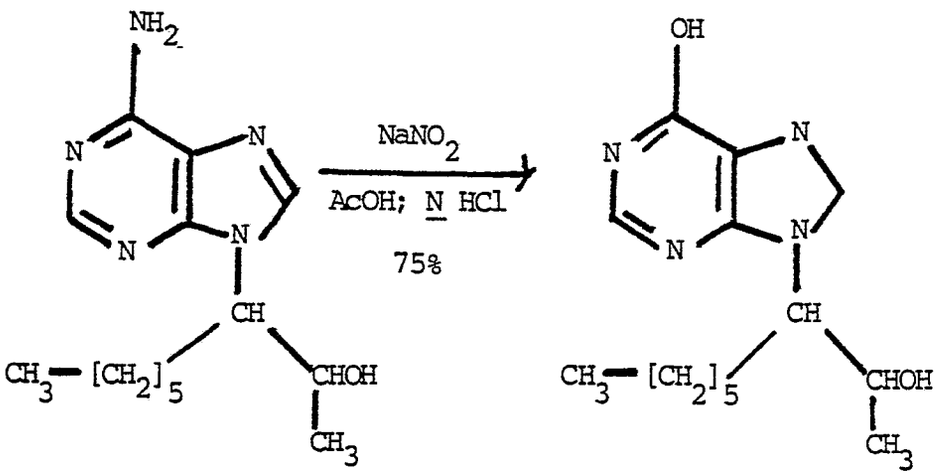
The crude oily erythro-9-(2-hydroxy-3-nonyl)-6-chloropurine (VII) (6.15 g) from the preceding preparations is dissolved in saturated methanolic ammonia (300 ml) and ammonium chloride (1 g) at 80-100° for 1 hr. in a stainless steel bomb (Parr Instruments). After cooling, the solution was evaporated to dryness in vacuo. Methanol was added and evaporated again (3 times) to eliminate the excess of ammonia.

The syrupy residue was dissolved in absolute methyl alcohol, and dry HCl gas was bubbled, keeping the temperature below 20° (with an ice water bath). After passing HCl for 1/2 hr., the mixture was cooled

at 5°. The precipitate was collected through a sintered glass funnel, washed with cold methyl alcohol and dried in air. Yield 6.0 g (92%) m.p. 173-175° dec. uv λmax 260 nm (in H_2O, pH 5.5).

ALTERNATE ROUTE FOR THE PREPARATION OF ERYTHRO-9-(2-HYDROXY-3-NONYL)HYPOXANTHINE (VIII)

(By deamination of VII)



VII VIII

Sodium nitrite (5.6 g, 71 mmole) was added slowly to a solution of erythro-9-(2-hydroxy-3-nonyl)-adenine (IX, 4.0 g, 14 mmole) in 50% acetic acid (20 ml) and N HCl (3.2 ml) at 25° with stirring. The mixture was stirred for 2 hr. at 25°. After this time,

UV spectrum is monitored. When UV max reached 250 mm, the solution was neutralized with 2 N NaOH. The resulting precipitate was filtered and washed with H_2O. Yield = 3.03 g (75%) m.p. = 195°.

An analytical sample was recrystallized (3x) from water yielding a product m.p. 202°. Anal. Calc. for $C_{14}H_{22}N_4O_2$: C, 60.40; H, 7.96; N, 20.13. Found: C, 60.40; H, 7.90; N, 20.12.

Method K

COMPOUND NPT 15426

There was used the procedure of H.J. Schaeffer and S.F. Schwender, J. Med. Chem. 17:6 (1974).

Method L

PREPARATION OF NPT 15410

0.1 mmoles of 9-(2-hydroxy-3-nonyl)-6-hydroxy purine, NPT 15392 (27.9 mg) and 0.3 mmoles of 2-hydroxypropyl, dimethylammonium 4-(acetylamino)benzoate (DIP·PAcBA) (77.1 mg) were accurately weighed and dissolved in 105 ml of 0.25% sodium carbonate ($NaCO_3$) to yield a 0.1% solution of NPT 15410 (the compound formed from NPT 15392 and (DIP·PAcBA) in a 1:3 molar ratio).

EVIDENCE FOR COMPLEX FORMATION

Phase solubility studies carried out with NPT 15392 and DIP·PAcBA demonstrate that NPT 15392 has increased solubility at increasing concentrations

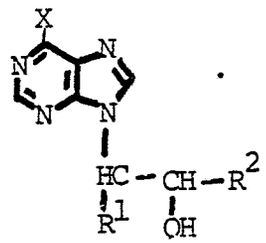
of DIP·PAcBA under conditions of constant pH. This is indicative of an interaction occurring in solution to yield a complex.

In place of the mole ratio of 1:3 (NPT 15392 and DIP·PAcBA), other complexes are formed by using mole ratios of 1:1 and 1:10.

Antiviral activity is shown in Tables 2 and 3.

Table 2

INHIBITION OF INFLUENZA VIRUS REPLICATION BY 9-(HYDROXYALKYL) PURINES



$\cdot (Y)_3$

Viral Strain: Influenza A USSR/90 (H_1N_1)

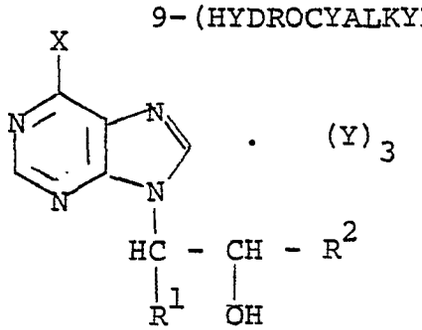
Test Cpd No.	Compound				%Inhibition of Hemadsorption Foci Conc. (μg/ml) Test Compound		
	R^1	R^2	X	Y	<10	10-100	>100
15425	H	H	OH	-	-	-	-
15428	H	H	OH	DIP·PAcBA	-	-	-
15435	H	H	SH	-		50	10
15437	H	H	SH	DIP·PAcBA		65	65
15446	H	CH_3	OH	-	2	0	0
15447	H	CH_3	OH	DIP·PAcBA	10	26	34
15431	H	CH_3	NH_2	-		22	0
15432	H	CH_3	NH_2	DIP·PAcBA		48	62
15427	CH_3	H	1	-			
15423	CH_3	H	Cl	-	2	13	6
15433	CH_3	H	NH_2	-		32	0
15434	CH_3	H	NH_2	DIP·PAcBA		41	62
15443	CH_3	H	OH	-		0	0
15444	CH_3	H	OH	DIP·PAcBA		44	54
15417	C_6H_{13}	H	OH	-	18	58	60

Table 2 (cont.)

Test Cpd No.	R^1	R^2	X	Y	<10	10-100	>100
15418	C_6H_{13}	H	OH	DIP·PAcBA	16	46	52
15392	C_6H_{13}	CH_3	OH	-	86	100	100
15410	C_6H_{13}	CH_3	OH	DIP·PAcBA	58	96	96
15426	C_6H_{13}	CH_3	NH_2	-	50	96	100
15110	-	-	-	DIP·PAcBA	0	0	20

Table 3

INHIBITION OF HERPES VIRUS REPLICATION BY 9-(HYDROCYALKYL) PURINES



NPT No.	Compound R^1	R^2	X	Y	Plaques (PFU) Test (15-150 g/ml)	Control	Percent Inhibition
15392	C_6H_{13}	CH_3	OH	-			98%
15417	C_6H_{13}	CH_3	OH				
15418	C_6H_{13}	H	OH	DIP·PAcBA			
15410	C_6H_{13}	H	OH	DIP·PAcBA			98%

BIOLOGICAL ACTIVITY

Methods

Anti-Influenza Activity - (Hemadsorption Assay)

Upon infection of a monolayer of tissue culture cells by influenza virus, the cell surface is altered so that guinea pig erythrocytes can be adsorbed to the cell surface. The number of foci of adsorbed cells (hemadsorption foci forming units HAFFU) is a quantitative measure of infectivity. The method is as follows.

The monolayers were subcultured in the following manner: The medium was poured off, and the monolayer washed two times with approximately 50 ml per wash of calcium and magnesium free phosphate buffered saline (PBS), (GIBCO #419) at a pH of 7.2. One ml of trypsin-EDTA solution (GIBCO #530L) containing 0.5 g trypsin(1:250) and 2.0 g EDTA/liter of Modified Puck's Saline A was added at 37°C to each flask and dispersed over the monolayer with gentle shaking. The flasks were then placed in an incubator at 37°C. for approximately 3-5 minutes depending on the time required to dislodge the cells. Occasional shaking was required. Ten ml of planting medium was added to each flask and the cells dispersed by aspirating and expelling the suspension from the pipette. The contents of a series of flasks were pooled and the cells in the suspension were diluted with planting medium to 7-8.5 x 10^4 cells/ml. The planting medium consisted of the following composition: Minimum Essential Medium Eagles (MEM) with Earle's salts and HEPES buffer (GIBCO #236) supplemented by adding the following substances as specified to 87 ml of MEM:

10 ml of fetal calf serum (FCS-GIBCO #614HI)

1 ml of L-glutamine (200 Molar-GIBCO #503)

1 ml of Chlortetracycline (5000 g/ml) GIBCO #528)

1 ml of 10,000 units penicillin, 10,000 g streptomycin and 10,000 neomycin mixture (PSN-GIBCO #564)

The cells were subcultured into Linbro tissue culture trays. The trays consisted of 24 flat bottom wells each with a 3 ml capacity per well; the cell culture suspension (1 ml) was added to each wall.

The following day the medium was removed and replaced with fresh planting medium. The monolayers were used for experimentation when they reached a condition in which they were almost confluent (approximately 3-4 days).

When the Linbro tray HeLa cell cultures were ready for experimentation (see cells), the medium was decanted and 1 ml of maintenance medium (MEM with FCS reduced to 3%) containing the compound being tested at a given concentration was added to 4 replicate cultures within a tray.

A series of different drug concentrations ranging from 2.3 to 150 g/ml were used. Maintenance medium alone was used for control cultures. After the administration of drug and control medium, 0.1 ml of the diluted viral suspension was added to experimental groups and infected control cultures. Saline alone

was added to non-infected control cultures. The Linbro trays were then incubated at 37°C. for 18 hours, after which media in all groups was aspirated. Each culture was washed once with PBS. The saline was aspirated and 0.5 ml of a 0.4% v/v guinea pig red blood cell suspension in PBS was added to each culture well. The cultures remained at room temperature for 30 minutes after which the medium was decanted and culture washed 2 times with PBS to remove all but the specifically bound red cells. After the third wash, maintenance medium was added to all cultures.

A Howard Micrometer eyepiece (C8385) was inserted within the ocular of a Nikon inverted phase contrast microscope. Each culture was scanned with a 4 x low paper objective and direct counts of hemadsorbed red cells were counted using the eyepiece grid as a field marker. Partial or complete fields were counted per experimental group depending on the resulting number and density of hemadsorbed cells in the infected control cultures. Magnification of 60 x or 150 x were chosen to obtain the best conditions for enumerating the hemadsorbed cells. Field factors were calculated for counting hemadsorption at 60 x and 150 x. At 60 x magnification, total field count was calculated using a multiplication factor of 55.5. At 150 x magnification the multiplication factor was 273. The multiplication factors of 55.5 and 273 represent the total number of fields at 60x and 150 x magnifications, respectively. The number of fields counted ranged from 3 to 5 per well with 3 to 4 wells per treatment group employed (see raw data tables in

results section for number of fields examined). Means and standard errors were calculated and the data was evaluated using student's t-test analysis.

BIOLOGICAL ACTIVITY

Anti-Herpes Activity - (Plaque Assay)

The infection of tissue culture cells by Herpes virus causes cell lysis. After a period of time these lysed cells are visualized as a tiny clear area (plaque) on a layer of cells. The incorporation of a test substance into the media will reduce the number of plaques if it is capable of preventing virus replication. The method is as follows:

MATERIALS AND METHODS

Virus

There was employed herpes hominis type 2 purchased from American Type Culture Collection (ATCC), Bethesda, Maryland, ATCC #VR 540, Lot 3D. The lypothilized viral suspension was reconstituted with 1 ml sterile distilled H_2O. The virus was passed twice through HeLa-cell monolayers. The tissue-culture supernates were pooled, dispensed in 1-ml aliquots, and stored at -70°C. The titer of this working-stock suspension was found to be 10^{-4} $TCID_{50}$/0.1 ml (2 days' incubation).

Herpes Virus Plaque Assay

Vero cells in log-growth phase were subcultured at a concentration of $1x10^5$ cells/ml in 50-ml Falcon flasks in Eagle's Minimum Essential Medium (MEM), supplemented with 10% fetal calf serum (FCS) and antibiotics. Media were changed the day following planting. The Vero monolayers reached confluence by the second day after planting and with the cells in log phase, the cultures were used for the plaque assay.

Culture media were poured off and the monolayers were washed once with phosphate-buffered saline (PBS). Several different dilutions of the working-stock virus suspension were prepared and each culture flask was infected with 0.5 ml of one of the virus dilutions added to FSC-free medium. This medium contained drug at a concentration of 150 µg/ml. Controls were prepared with medium devoid of drug.

Virus adsorption was allowed to proceed for 2 hours at 37°C, during which time the cultures were rocked gently every 15 minutes. Then Media were poured off and the monolayers were washed once with 10 ml PBS.

Agarose was prepared at a concentration of 6% w/v in 50 ml PBS. A stock medium of MEM supplemented with 2% FCS was prepared. Drug was added to some of the stock medium at 150 µg/ml. The three solutions were maintained at 47°C. In addition, a 1:10 dilution of pooled human anti-herpes sera was readied. Just before the start of treatment, 15 ml of the agarose solution was added to 85 ml of medium. Another 15 ml of agarose were added to 85 ml of drug-medium.

Each of the washed monolayers in one group of experiments was treated either with 5 ml of agarose-medium or with 5 ml of agarose-drug-medium. In another group of experiments, each monolayer was treated either with 0.2 ml of anti-herpes sera in 5 ml of stock medium, or with 0.2 ml of anti-herpes sera in 5 ml of drug-medium. The anti-herpes sera were used in place of agarose to localize plaques by neutralizing any free virus in the medium. The flasks were allowed to remain at room temperature for 5 minutes, after which they were incubated at 37°C for 2 days. Triplicate cultures were used for most treatment groups.

Ten ml of PBS then were added to each flask. Overlays were shaken gently and then were poured out of the flasks. The monolayers were stained with a solution 0.5% w/v crystal violet in 50% methanol in triple-distilled H_2O.

Plaques were counted either directly by transmitted fluorescent light and macroviewing, or by the use of light microscopy for microplaques. Microplaques were counted by averaging three fields per experimental group under 150x magnification.

In other tests of antiviral activity the following results were obtained.

Compound	% Inhibition at µg/ml				Virus
	0.1-1.0	1.0-10	10-100	>100	
15392	30-50	--	>70	>70	Influenza A Swine 1976 (H_{1sw}-N_1)
15417	--	50-70	>70	--	Influenza A Swine 1976 (H_{1sw}-N_1)
15418	--	--	>70	>70	Influenza A Swine 1976 (H_{1sw}-N_1)
15426	20-30	30-50	50-70	50-70	(Russian)
15410	50-70	30-50	>70	>70	(Swine)

Additional antiviral activity tests of Compound NPT 15410 are shown in Table 3a.

Table 3a

INHIBITION OF INFLUENZA VIRUS

REPLICATION BY NPT 15410

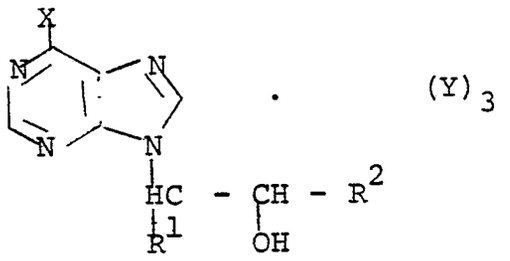
Virus	Concentration Range (µg/ml)			
	.01-1.0	1.0-10.0	10.0-100	>100
A/Swine/76 ($H_{1sw}N_1$)	+++[a]	++	++++	++++
A/Texas/77 (H_3N_2)	++	+	++++	++++
A/Dunedin/73 (H_3N_2)	NT	NT	±	++
A/Jap/305 (H_2N_2)	NT	NT	++++	++++
A/PR_8 (H_0N_1)	++	++	++++	+++
A_2 Hong Kong (H_2N_2)	NT	NT	++	+++

[a]
NT = Not tested
± = 10-20% Inhibition
+ = 20-30% Inhibition
++ = 30-50% Inhibition
+++ = 50-70% Inhibition
++++ = >70% Inhibition

Immunomodulation activity is shown in Table 4.

Table 4

MODULATION OF CELL MEDIATED IMMUNITY BY 9-(HYDROXYALKYL) PURINES



	Compound			
NPT No.	R^1	R^2	X	Y
15425	H	H	OH	-
15428	H	H	OH	DIP·PAcBa
15435	H	H	SH	-
15437	H	H	SH	DIP·PAcBA
15446	H	CH_3	OH	-
15447	H	CH_3	OH	DIP·PAcBA
15431	H	CH_3	NH_2	-
15432	H	CH_3	NH_2	DIP·PAcBA
15427	CH_3	H	1	-
15423	CH_3	H	CL	-
15433	CH_3	H	NH_2	-
15434	CH_3	H	NH_2	DIP·PAcBA
15443	CH_3	H	OH	-
15444	CH_3	H	OH	DIP·PAcBA
15417	C_6H_{13}	H	OH	-
15418	C_6H_{13}	H	OH	DIP·PAcBA
15392	C_6H_{13}	CH_3	OH	-
15410	C_6H_{13}	CH_3	OH	DIP·PAcBA
15426	C_6H_{13}	CH_3	NH_2	-

Maximum Percent Change

NPT No.	Mitogen Induced (Con A) Mouse Lymph. Prolif.			Mitogen Induced (PHA) Human Lymp. Prolif.		
	.01-1.0	1.0-10	10-100	.01-1.0	1.0-10	10-100
15425	11	0		100	0	0
15428						
15435				55	45	-50
15437						
15446	6	12	0	0	0	0
15447						
15431	-15	-27	-47	0	0	0
15432						
15427						
15423						
15433	+15	-23	-65	53	0	-50
15434						
15443	+17	+27	+27	0	+13	0
15444						
15417	0	0	0	0	0	-50
15418	41	73	26			
15392	172	162	-72	11-15	20	-50
15410	140	40	40	30-50	60	
15426	-50	-85	-91	6	-41	

Maximum Percent Change (Cont.)

NPT No.	Lymphokine Induced (MMF) Guinea Pig Mac. Prolif. .01-1.0	1.0-10	10-100
15425			
15428			
15435			
15437			
15446	0	0	0
15447			
15431			
15432			
15427			
15423			
15433			
15434			
15443	+20	0	
15444			
15417		13	-50
15418	12	80	-50
15392	33	23	
15410	12	23	
15426			

Several compounds were tested for Mitogen Induced Murine Lymphocyte Proliferation with the following results:

Compound	% Stimulation at µg/ml			
	01-1.0	1.0-10	10-100	>100
15392	>50%	30-50%	30-50%	not tested
15426	0	0	0	not tested
15410	>50	30-50	30-50	not tested
15417	0	0	0	0
15418	20-30	>50	20-30	not tested

BIOLOGICAL ACTIVITY

Immunomodulating Assay

The following three assay procedures are used to evaluate the ability of the test substances to modulate the activity of several classes of cells in the immune system. In these systems it is possible to identify both immunopotentiating activity (evidence by an enhancement of the parameter examined) as well as immunosuppressant activity (evidenced by an inhibition of the parameter examined).

1. Mitogen-Induced Mouse Spleen Cell Assay

Mouse spleen cells contain a population of both B and T lymphocytes which can be stimulated by a number of foreign substances (e.g., plant mitogens such as Con A) to proliferate. This enhanced proliferation is an indication of enhanced cell mediated immunity. The method below describes the system used to evaluate test substances as immunopotentiators.

MATERIALS

Concanavalin A (Calbiochem, La Jolla, California), Lot #210073, lyophilized in NaCl, was prepared first as a 1% solution and diluted as a 2X concentration for each dilution (0.5, 1.0, 2.5 µg/ml).

Animals

Six to eight week old male Balb/c and C_3H inbred mice were obtained from the following sources: Flow research Animals, Inc., Dublin, Virginia; Charles River Breeding Laboratories, Wilmington, Massachusetts; Laboratory Supply Company, Indianapolis, Indiana; and Lionel Strong Foundation, San Diego, California.

Cells

Three to five mice were sacrificed by cervical dislocation and the spleens aseptically removed. Pooled spleens were minced and teased with sterile forceps; then strained through a double layer of nylon mesh. The cell suspension was washed once with 15 ml of RPMI 1640 supplemented with 5% fetal calf serum and antibiotics. Cells were cultured at a concentration of 10^6 cells/0.1 ml/well in micro-plates. Cultures

were incubated in the presence or absence of mitogen in a humidified atmosphere containing 5% CO_2 for 48 hours. The test compound was added to cultures at various concentrations concommitant with mitogen.

Proliferation

Proliferation was assayed by the degree of incorporation of 1.0 Ci of [^{3}H] thymidine over an 18 hour incubation period. Cultures were harvested by a MASH unit (Otto Hiller Co., Madison, Wisconsin) and thymidine incorporation was assayed by liquid scintillation spectrometry. Cultures were performed in triplicate and data are expressed as means plus or minus the standard error of the experimental means. Drug stimulation indices over control values were also calculated and portrayed graphically.

2. Mitogen Induced Human Peripheral Blood Lymphocytes - A clinical need exists for therapeutic agents to augment the immune response in patients with deficient or depressed immune states, such as exists in viral diseases or cancer. By studying the ability of agents to augment the proliferation of human peripheral blood lymphocytes in response to a foreign substance one can identify agents with immunopotentiating activity in man. The procedure is that just set forth and that also described by Hadden, J.W., Infect. & Immunity, February, 1976, pages 382-387, especially pages 382-383.

3. The macrophage represents a subpopulation of white blood cells which is an important component of the immune system in control of both cellular and humoral immunity. The assay system described below evaluates the substances studied as potentiators of macrophage function.

Phytohemagglutinin (PHA) (HA-17) was purchased from Burroughs Wellcome. A preparation containing Macrophage Mitogen Factor (MMF) and Macrophage Activating Factor (MAF) was prepared from antigen-stimulated immune lymph node lymphocytes (guinea pig) as previously described by Hadden et al, Nature 257, 483-485 (1975). Partial purification of this preparation by vacuum dialysis and sephadex G-100 column chromatography yielded an active fraction in the range of 35-70,000 daltons exhibiting both mitogenic and activating properties. The active fraction was employed in both the proliferation and activation assays.

Methods

Ficoll-hypaque purified human peripheral blood lymphocytes were prepared and PHA-induced lymphocyte proliferation was assayed by the incorporation of tritiated thymidine as described in Hadden et al, Cell. Immunol. 20, 98-103 (1975). Each compound was analyzed in the presence of suboptimal, optimal and supraoptimal concentrations of PHA (.001, .01, 0.1 units/ml respectively). Parafin oil-induced guinea pig peritoneal macrophages were prepared and incubated as monolayer culture (>98% pure macrophages). Lymphokine (MMF)-induced proliferation was assayed by the

incorporation of tritiated thymidine at 3 and 5 days of culture as described, Hadden et al, Nature 257, 483-485 (1975). Lymphokine (MAF)-induced macrophage activation to kill Listeria monocytogenes following 5 days of culture in the presence or absence of MAF was performed during a 6 hour period as described in Hadden and England, Immunopharmacology, pages 87-100 (Plenum Press, 1977). Phagocytosis was quantitated during a 20-minute exposure to Listeria monocytogenes by counting the number of macrophages containing bacteria and the number of bacteria per phagocytic cell on gram stained monolayers in Labtek chambers. Intracellular killing of bacteria was evaluated by counting the number of cells containing bacteria and the number of bacteria/cell 6 hours after the initial 20 minute exposure. Parallel experiments in which macrophages were lysed and intracellular bacteria were cultured confirm the validity of bacterial activity determined by this manner in this system. The drugs were employed in each of the three systems over serial log concentration range in triplicate in the presence and absence of mitogen or lymphokine. Each type of experiment was performed at least three times. Previous experiments indicate a parallelism of response to pharmacologic modulation in the proliferation and activation assays.

BIOLOGICAL ACTIVITY

Anti-Leukemic Activity (Inhibition of L-1210 Growth

Leukemic cells isolated from mice bearing the L-1210 tumor are cultured *in vitro* and their growth can be measured by counting the number of cells in the culture over a period of time. The incorporation of a test substance into the media will prevent the growth of the leukemic cells, an indication of an effective anti-leukemic agent.

I_{50} (concentration of drug inhibiting growth of L-1210) by % for the tested compounds was as follows:

Compound	Concentration (micrograms/ml)
15392	28
15410	54
15417	47
15418	70

The assay system used is set forth below.

To Measure Inhibition of Leukemic Cell (L-1210) Growth

Check to see that there is adequate cell growth in the stock cultures. Use cells 48-72 hours after transfers are done.

Weigh out the drugs at 50 times the desired final concentration and made serial dilutions.

Make up the final medium using 500 mls McCoy's 5A medium, 15% fetal calf serum, 5 mls penicillin-streptomycin solution, and 5 mls antibiotic-antimycotic solution and let it stand at room temperature.

Using sterile technique, add 0.1 ml of the drug dilutions to each tube.

Add an appropriate quantity of cells to the prepared medium. After mixing, remove a 0.5 ml sample, place it in a vial containing 9.5 mls of saline, and count it on the Coulter Counter. Multiply the count by 40 to compensate for the 40 fold dilution (0.5 ml into 0.5 ml saline and record the inoculum).

Add 5 mls of cell suspension to each tube. Swirl the bottle every 4 tubes to insure a more uniform distribution of cells.

Tighten the caps and place in the CO_2 incubator at 36-38° for 96 hours.

After 96 hours remove the tubes from the incubator and count the contents of each on the Coulter Counter. Multiply all counts by 40 and average the four counts for each drug dilution. If the count is less than the inoculum, record 100% inhibition. If the count is greater than the average of the eight control counts, record 0% inhibition. For all other counts use the following formula:

$$\frac{\text{Average cells/ml in treated cultures - inoculum in cells/ml}}{\text{Average cells/ml in control cultures - inoculum in cells/ml}} \times 100 = \% \text{ Survival}$$

100% - % survival = inhibition of growth due to treatment.

The subject compounds of this invention have been shown to inhibit the replication of a representative sample of both RNA and DNA viruses using standard tissue culture techniques. In the case of the RNA viruses, several strains of influenza virus belonging to both the A and B sub-types were shown to be inhibited, using the hemadsorption technique (Section II, B). The specific compounds found to inhibit influenza virus replication (Type A/USSR 90) are shown in Table 1. Several members of the Series NPT 15392, NPT 15410, NPT 15417, and NPT 15418 were shown to inhibit the replication of at least 4 different strains of influenza virus at concentrations ranging from 1-150 g/ml.

In addition, several members of the Series, NPT 15410 and 15392, have been shown to inhibit the replication of Herpes Simplex virus, a member of the DNA class of viruses and a virus responsible for severe mucocutaneous lesions in man, along with the fatal Herpes encephalitis. Other members of this class of viruses are responsible for hoof and mouth disease in swine and cattle and infections rhinotracheitis in cats and kennel cough in dogs. Even

concentrations less than 100 µg/ml of NPT 15392 and 15410 were found to reduce plaque formation caused by Herpes Simplex virus to an extent of >90%. Other members of the RNA and DNA class of viruses are shown in Table 5 and are responsible for the diseases specified. Of all the diseases in the world at least 25% are known to be caused by viruses. In addition, a number of viruses have been isolated that are shown to produce tumors. Thus, antiviral agents may be expected to, by themselves, have some antitumor properties.

It is an established fact that many infectious agents, such as viruses (influenza virus, HSV, Friend leukemia virus), bacteria and fungi cause an immune suppressed state in the host, weakening his defenses to infection by infectious agents. Most other antiviral antimetabolite substances, like AraC, cause a suppression of host immune defense mechanisms, thereby exhibiting potential to lessen the body's own natural defense mechanisms and enhance secondary infection.

An immunopotentiator or immunomodulator is any agent which either restores depressed immune function, or enhances normal immune function, or both. Immune function is defined as the development and expression of humoral (antibody-mediated) immunity, cellular (thymocyte-mediated) immunity, or macrophage and granulocyte mediated resistance. It logically includes agents acting directly on the cells involved in the expression of immune response, or on cellular or molecular mechanisms which, in turn, act to modify

the function of cells involved in immune response. Augmentation of immune function may result from the action of an agent to abrogate suppressive mechanisms derived by negative-feedback influences endogenous or exogenous to the immune system. Thus, immune potentiators have diverse mechanisms of action. Despite the diversity of cell site of action and biochemical mechanism of action of immunopotentiators, their applications are essentially the same; that is, to enhance host resistance.

Applications of Immunopotentiators

1) The principal protective function of the immune system relates to resistance to invasion by pathogens, including viruses, rickettsia, mycoplasma, bacteria, fungi, and parasites of all types. Thus, improvement of immune response, particularly when depressed, would calculatedly improve resistance in infection or infestation by any of the above pathogens. An immunopotentiator alone or in combination with anti-infective therapy can be applied to any and all infectious diseases.

2) A second protective function of the immune system is thought to be resistance to engraftment of foreign tissue, either natural as in the fetal-maternal relationship; or unnatural as performed by the transplant physician. Immunopotentiators can also be used to facilitate rejection of fetal or placental tissues or to modify or induce tolerance to grafts.

3) A third protective function of the immune system is thought to be resistance to malignant cell development as in cancer. The use of immunopotentiators can be used in cancer treatment to enhance tumor rejection and to inhibit tumor recurrences following other forms of therapy.

4) A fourth protective function involves the capacity to recognize foreign-ness and to maintain non-reactivity to self by positive suppressor mechanisms. In auto-immune and related disorders, immune reactivity directed at self antigens or exaggerated, elevated responses are apparent which are self-destructive. Immunopotentiators can be used to restore normal suppressor mechanisms, induce tolerance, or otherwise promote a normal immune response.

Each of the protective functions of the immune system can be modified by non-specific therapy with immunopotentiators alone or in combination with other agents employed to improve resistance or to kill the invading pathogen. In addition, specific resistance can be augmented by use of immunopotentiators in conjunction with some form of antigen as in a vaccine employing, for example, virus, tumor cell, etc. This use can be to induce either specific immunity or tolerance. The latter might be exemplified by use with antigen in allergy or auto-immune diseases. Use of immunopotentiators may be either therapeutic or prophylactic; the latter particularly in aging, where infection, auto-immunity, and cancer are more common. The timing of administration and routes are variable and may be critical in determining whether a positive

or negative response results. Any agent capable of augmenting immune response may inhibit it depending on timing and dose; thus, under certain circumstances an immunopotentiator could be used as an immunosuppressive agent for use in allergy, auto-immunity and transplantation.

Table 4 above presents the results of an evaluation of a number of these subject compounds as potentiators of the immune response. Three different test systems were used. The first involves a measure of the ability of the test compound to enhance the ability of mouse lymphocytes to proliferate in response to a plant mitogen (Con A). The second involves measuring the ability of the test compounds to enhance human lymphocyte proliferation in response to a second plant mitogen (PHA). The third system measures the ability of these test substances to enhance macrophage proliferation in response to a natural lymphokine (MMF, Macrophage Mitogenic Factor). This latter response, the proliferation and activation of macrophages, has been shown to be involved in the killing of bacteria, viruses and tumor cells by this class of white blood cells.

Significant potentiation of the immune response has been observed by 15392, 15410, and 15418.

Finally, the activity of several of these agents, NPT 15392 and 15410 as inhibitors of the growth of abnormal lymphocytes has been determined. Notably, both substances are capable of inhibiting the proliferation of mouse leukemic lymphocytes (an L-1210

cell line) in tissue culture. A 50% inhibition of L-1210 cells was effected by NPT 15392 at 28 µg/ml and by NPT 15410 at 54 µg/ml. The ability to inhibit leukemic lymphocytes at concentrations that stimulate normal lymphocytes is a unique property not known to be present in any other class of substances.

The products of the present invention are members of a class of substances, which specifically inhibit the replication of RNA and DNA virus, modulate (potentiate) the immune response and inhibit the growth of leukemic lymphocytes. Based on in vitro experiments, which demonstrate activity over a concentration range of 0.01-150 µg/ml, dose ranges effective in mammals are 0.05-500 mg/kg. A lack of toxicity has been noted at levels of 1,500 mg/kg in mice for certain numbers of this series.

The immunopotentiators of the invention can be employed, for example, to provide resistance to invasion by the viruses in Table 5.

Table 5

Virus	Class	Disease
Arenavirus	RNA	Rift Valley Fever
Influenza	RNA	Influenza
Rhinovirus	RNA	Common Cold
Poliovirus	RNA	Polio
Measles	RNA	Rubella
Newcastles Disease Virus	RNA	Newcastles disease
Rotavirus	RNA	Gastroenteritis in infants
Hepatitis Type A	RNA	Infectious Hepatitis
Rabies virus	RNA	Rabies
Arbovirus	RNA	Encephalitis
Vaccinia virus	DNA	Smallpox
Herpes Simplex Virus	DNA	Cold sore, Encephalitis, Venereal Disease
Herpes Zoster	DNA	Shingles
Varicella Zoster	DNA	Chicken pox
Adenovirus	DNA	Respiratory
Hepatitis Type B	DNA	Chronic Hepatitis, Severe Hepatitis
Hoof and Mouth Disease virus	DNA	Hoof and Mouth Disease
Machupo Virus		Hemorrhagic Fever

POTENTIATION BY DIP PAcBA OF BIOLOGICAL ACTIVITIES

Of the substances described in Table 1, NPT 15392 and NPT 15446 are new compounds claimed in the application of Alfredo Giner-Sorolla filed on even date. Also new are the DIP·PAcBA salts presented in this table, namely 15428, 15437, 15447, 15432, 15434, 15444, 15418 and 15410. NPT 15392, NPT 15417, NPT 15426 have all been shown to have significant anti-influenza activity by themselves. In one instance (with NPT 15392) the addition of DIP·PAcBA salt to NPT 15392 to form 15410 does not potentiate the anti-influenza activity. In the case of NPT 15417, addition of DIP·PAcBA salt to form 15418 does potentiate the anti-influenza activity. A summary of the relative ability of DIP·PAcBA salts to potentiate the different biological activities is set forth below.

Table 6

Compound	DIP·PAcBA Salt	Potentiation Anti-Influenza	Anti-Leukemia	Immuno-potentiation
15392	15410	both are equally active	Yes	Yes
15417	15418	Yes	-	Yes
15435	15437	Yes	-	-
15446	15447	Yes	-	-
15431	15432	Yes	-	-
15433	15434	Yes	-	-
15443	15444	Yes	-	-

FORMULATIONS

The compounds of the present invention can be fed to a mammal at a dosage of 1-1000 mg/kg of body weight and are believed to be active at levels as low as 0.05 mg/kg. The LD_{50} as determined in mice of NPT 15410 given intraparenterally was 4,300 mg/kg, while subcutaneously was 4,900 mg/kg. NPT 15392 has been given to mice at doses of 1000 mg/kg and no drug related mortality was noted.

They can be administered in tablet or capsule form to humans and where solubility permits in the form of syrups or injectable solutions or where insoluble as suspensions. Typical pharmaceutical formulations are described below:

Capsule:

NPT 15392	50-500 mg.
Avicel pH 101 (microcrystalline cellulose)	to make 800 mg.

Suspension:

Aqueous suspensions can be made with a number of suspending agents incorporated with the active drug substances. Included as suspending agents are such substances as sodium carboxymethylcellulose, Na alginate, gum tragacanth, Avicel RC-591 (microcellulose), methylcellulose, Veegum, Xanthan gum. In addition to a suspending agent such substances as sweeteners, flavors, colorants, preservatives, protective colloids and dispersants may be added.

TABLET FORMULATION

NPT 15392	50-500 mg
Avicel pH 101	130 mg
Starch, modified	20 mg
Magnesiumm stearate U.S.P.	5.5 mg
Polivinylpyrrolidone	22 mg
Stearic acid U.S.P.	30 mg

SYRUP FORMULATION

NPT 15392	25-125 mg	(or at maximum level of solubility)
Corn Sugar	3.25 g	
Distilled Water	.05 g	
FD and C Red 40	.00175 g	
Sodium Saccharin	.00250 g	
Alcohol U.S.P.	.08 g	
Methyl paraben U.S.P.	.005 g	
Propyl paraben U.S.P.	.001 g	
Glycerin	.31225 g	
Cherry flavor	.00825 g	
Fruit flavor	.00825 g	
Distilled Water g.s.ad	5 ml	

IN VIVO TREATMENT OF MICE WITH NPT 15392 AND NPY 15410: EFFECT ON THE IN VITRO STIMULATION OF SPLEEN CELL PROLIFERATION BY CONCANAVALIN A

The purpose of this study was to determine the effects of *in vivo* treatment of mice with the compounds NPT 15392 and 15410 on the subsequent activity of spleen cells isolated from these animals and evaluated *in vitro* for their proliferative response to the mitogen, Concanavalin A (Con A).

PROCEDURE

In Vivo Treatment

Nine male Balb/C mice, 8-9 weeks old, weighing 18-20 gms were divided into three groups. One group was treated twice daily (for 1 day), in the morning and afternoon, with an oral dose of NPT 15392 at 10 mg/kg. The second group was similarly treated with NPT 15410 at 20 mg/kg. A third group, dosed with saline served as a placebo control.

In Vitro Spleen Cell Assay: Cell Preparation

The following day, each group was sacrificed and the spleens removed and pooled. The spleens were minced and the cells washed in RPMI-1640 medium (Grand

Island Biologicals) supplemental with 2 mm glutamine and antibiotics. The cell concentration of each preparation was determined by a Coulter counter and adjusted to 5 x 10^6 cells/ml with RPMI medium.

Microtiter Plate Assay

Microtiter assays were carried out in 0.2 ml incubations, containing 5 x 10^5 cells and Con A or Con A and compounds at the indicated concentrations. All assays were performed with 6 replicates and compared with a blank assay containing only cells. The assay plates were incubated at 37° in 5% CO_2 for 4 days. During the final 18-20 hours of incubation, 0.5 ml of ^{3}HTdR (10 µCi/ml, 6 C_i/m mole) were added to each culture. The cultures were harvested with a multiple automatic sample harvester (MASH) unit and the incorporated ^{3}HTdR determined with a Beckman LS 8000 liquid scintillation counter, as a measure of cell proliferation. The results are tabulated as the ratio of the activity in the Con A or Con A and compound treated cultures to the blank cultures.

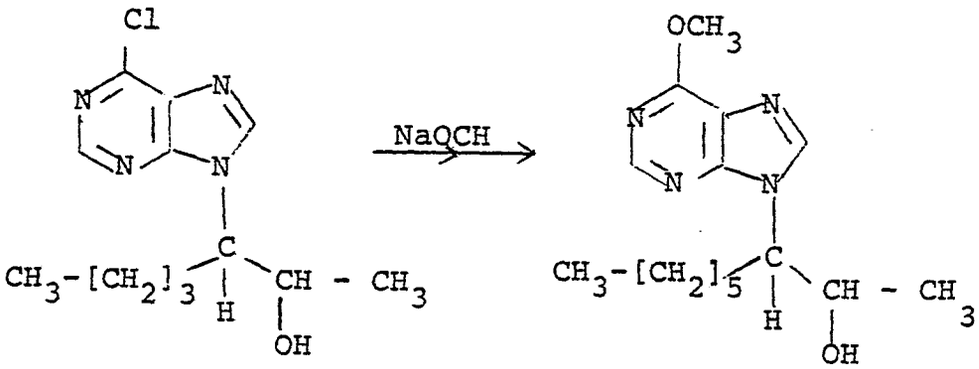
In vivo treatment with either compound 15392 or 15410 increases the subsequent response of the spleen cells, in vitro, to Con A stimulation at a suboptimal mitogen concentration (5 µg/ml). Thus compound 15410 increased the stimulation ratio to 100:1 compared to 55:1 with the placebo. No significant differences are obtained with either compound 15392 or 15410 treatment when the cells are stimulated with a more optimal concentration of Con A (10 µg/ml).

There was also tested the effect of subsequent in vitro treatment of Con A stimulated cells with NPT 15392 and 15410 at 1 µg/ml. Both compounds show a marked ability to augment the Con A stimulation, particularly at the suboptimal mitogen concentration (5 µg/ml) and to a lesser extent at 10 g/ml. AT 5 µg/l of Con A, the stimulation by NPT 15392 is 2.8 fold over Con A alone, while that for NPT 15410 is 3.3 fold.

These results indicate an immunomodulating effect of these compounds on spleen cell proliferation. Pre-treatment of animals with either compounds which sensitize the cells to subsequent mitogenic stimulation while exposure of the cells in vitro to the compounds following mitogenic stimulation will augment the proliferative response particularly under conditions when the response to mitogen alone is low.

EXAMPLE

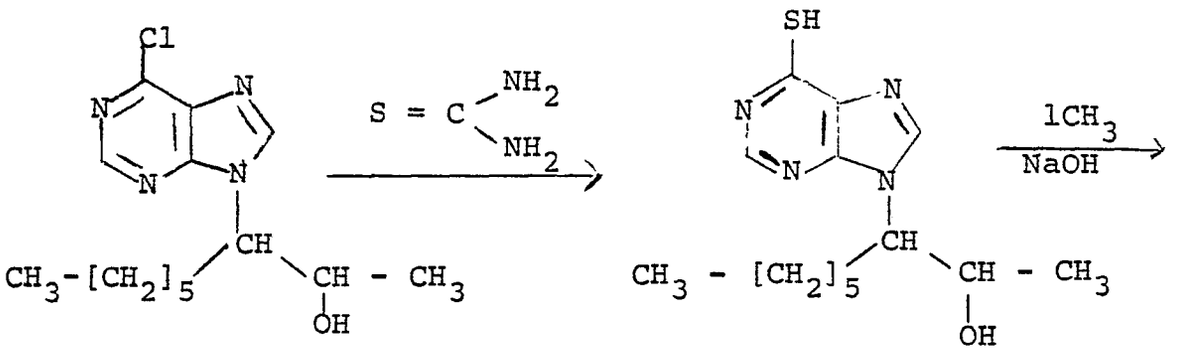
SYNTHESIS OF A ERYTHRO-9-(2-HYDROXY-3-NONYL) 6-ALKOXY PURINE (II)



I II

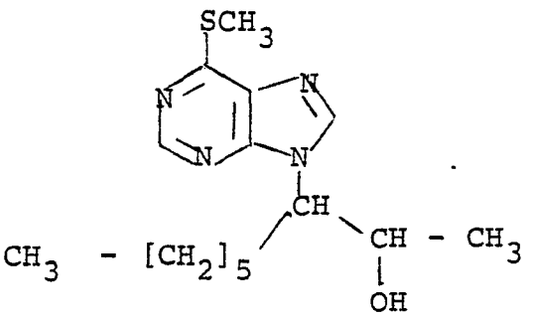
Compound I (10 mM) and a solution of sodium methoxide (11 mM) in methanol (50 ml) was refluxed for 6 hrs. The reaction flask was cooled, the pH adjusted to 5 with glacial acetic acid and the mixture, evaporated to dryness under reduced pressure. The residue was taken up with a minimum amount of cold water, filtered and dried in vacuo.

SYNTHESIS OF ERYTHRO-9-(2-HYDROXY-3-NONYL)-6-METHYLMERCAPTO PURINE (III)



I

II



III

Step (1) Compound I ⟶ Compound II

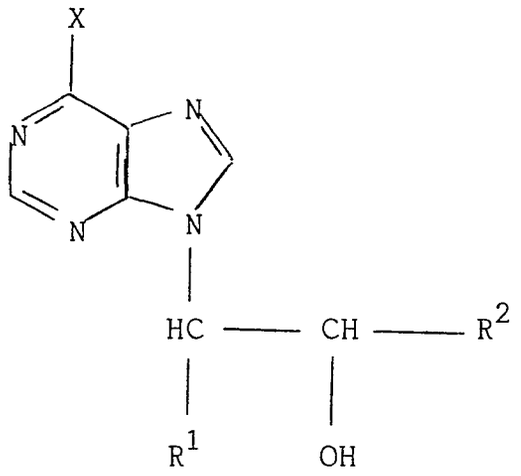
Compound I (10 mM) in ethanol (25 ml) and thiourea (10 mM) and anhydrous sodium acetate (11 mM) was refluxed for 1 hour. After cooling the resulting product was collected by filtration, suspended in minimum amount of cold water and the pH adjusted to 5 with diluted (20%) acetic acid. The product is washed with minimum amount of cold water, filtered and the precipitate dried under vacuo.

Step (2) Compound II ⟶ Compound III

A solution of Compound II (10 mM) in a 2 N NaClH (25 ml) was cooled at 5°. Methyl iodide (20 mM) was added and the mixture shaken vigorously in a tightly stoppered flask for 15 minutes, at 5°. The mixture was then mechanically stirred at room temperature (25°) for 3 hours, the pH adjusted to 5 with glacial acetic acid. The resulting precipitate was collected by filtration and washed twice with cold water (15 ml) and dried.

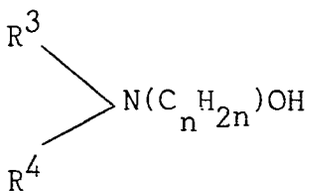
WHAT IS CLAIMED IS:

1. Complexes of 9-hydroxyalkyl-purines of the formula



$(Y)_z$

where X is OH, NH_2, SH, OR or SR (where R is alkyl of 1 to 4 carbon atoms or benzyl), R^1 is H or alkyl of 1 to 8 carbon atoms, R^2 is H or methyl, Y is the salt of an amine of the formula



where R^3 and R^4 are lower alkyl, n is an integer from 2 to 4 with p-acetamidobenzoic acid and where z is a number from 0 to 10.

2. A compound according to claim 1 where x is OH.

3. A compound according to claim 1 where X is NH_2.

4. A compound according to claim 1 where X is SH.

5. A compound according to claim 1 where R^1 is n-hexyl, R^2 is methyl, X is OH, NH_2 or SH and Y is the salt of dimethylaminoisopropanol and p-acetamidobenzoic acid.

6. A compound according to claim 1 where X is OH, R^1 is n-hexyl, R^2 is methyl, z is 3 and Y is the salt of dimethylaminoisopropanol with p-acetamidobenzoic acid.

7. Process for preparing the compounds according to claim 1, characterized in that

a) the corresponding 9-substituted 6-amino-purine is diazotized with sodium nitrite in acid solution to introduce a HO-group in 6-position

b) the corresponding 9-substituted 6-chloro-purine is treated with ammonia in alcoholic solution to introduce a NH_2-group in 6-position

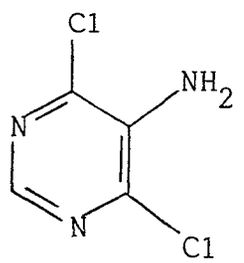
c) the corresponding 9-substituted 6-chloro-purine is treated with thiourea to introduce a SH-group in 6-position

d) the corresponding 9-substituted 6-chloro-purine is hydrolyzed in alkaline solution under heating to introduce an OH-group in 6-position

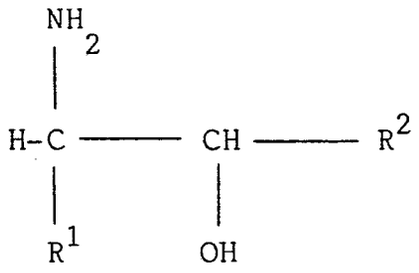
e) the corresponding 9-substituted 6-hydroxy-purine is reacted with an alkali alkoxide to introduce an alkoxy group in 6-position

f) the corresponding 9-substituted 6-mercapto-purine is reacted with an alkyljodide to introduce a thioalkyl group in 6-position, or

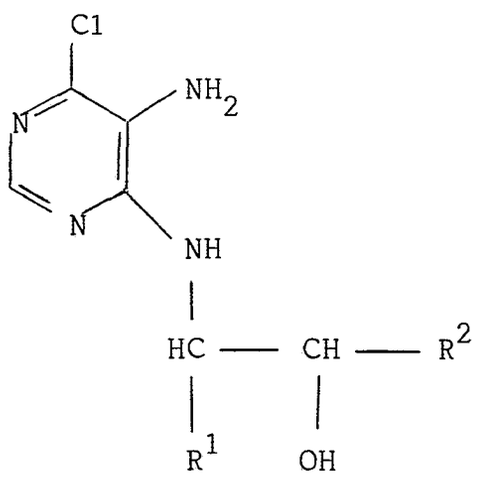
g) 5-amino-4,6-dichloro-pyrimidine of the formula



is treated with an amine of the generic formula



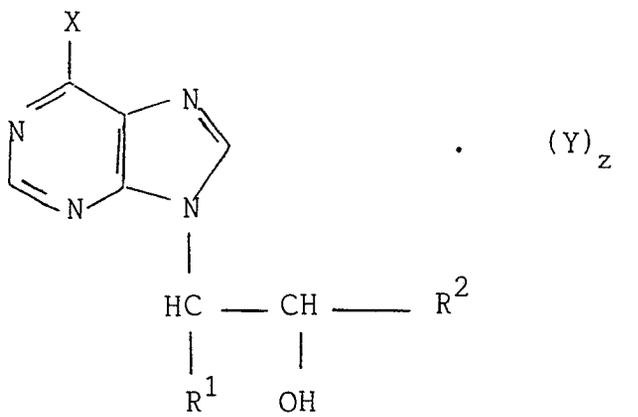
wherein R^1 and R^2 have the above meaning, and the obtained 4-chloro -5-amino-6-(hydroxyalkylamino)-pyrimidine of the generic formula



wherein R^1 and R^2 have the indicated meaning is subjected to a ring closure by treatment with triethylorthoformate, whereupon the obtained 6-chloro-9-hydroxylakyl-purine is subjected to one of process steps b), c) or d), and if desired, obtained 6-hydroxypurines according to process step e) are converted in 6-alkoxy-purines, while 6-mercapto-

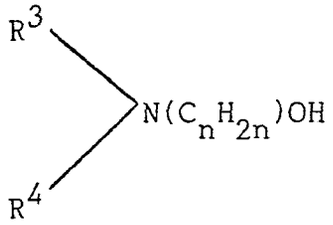
purines according to process step f) are converted in 6-thioalkyl-purines, a mixture is prepared from the compounds obtained according to one of steps a) to g) and from the salt Y in the mole ratio 1:1 up to 1:10, the mixture is dissolved and the formed complex is recovered from the solution.

8. Therapeutical composition for imparting immunomodulating, antiviral, antitumor or enzyme inhibiting activity, characterized in that it contains a compound of the generic formula



. $(Y)_z$

where X is OH, NH_2, SH, OR or SR (where R is alkyl of 1 to 4 carbon atoms or benzyl), R^1 is H or alkyl of 1 to 8 carbon atoms, R^2 is H or methyl, Y is the salt of an amine of the formula



where R^3 and R^4 are lower alkyl, n is an integer from 2 to 4 with a pharmaceutically acceptable acid and where z is a number from O to 10.

9. Composition according to claim 8 whereein z in the active ingredient is 1 to 10, X is OH, NH_2 or SH, R^1 is H or n-alkyl of 1 to 8 carbon atoms, R^3 and R^4 are alkyl of 1 to 4 carbon atoms and Y is the salt of dimethylamino-isopropanol and p-acetamidobenzoic acid.

10. Composition according to claim 9 wherein R^1 is n-hexyl and R^2 is methyl.

11. Composition according to claim 8 wherein X is OH, R^1 is alkyl of 1 to 8 carbon atoms and R^2 is methyl.

European Patent Office

EUROPEAN SEARCH REPORT

Application number
0009154
EP 79 10 3232

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim
D,A	JOURNAL OF MEDICINAL CHEMISTRY, vol. 8, July 1965, pages 502-506 Columbus, Ohio, U.S.A. H.J. SCHAEFFER et al.: "Enzyme Inhibitors. VIII. Studies on the Mode of Binding of Some 6-Substituted 9-(Hydroxyalkyl)purines to Adenosine Deaminase" * Pages 502-506 *	
D,A	JOURNAL OF MEDICINAL CHEMISTRY, vol. 17, no. 1, January 1974, pages 6-8 Columbus, Ohio, U.S.A. H.J. SCHAEFFER et al.: "Enzyme Inhibitors. 26. Bridging Hydrophobic and Hydrophilic Regions on Adenosine Deaminase with Some 9-(2-Hydroxy-3-alkyl)adenines" * Pages 6-8 *	
D,A	JOURNAL OF MEDICINAL CHEMISTRY, vol. 15, no. 5, May 1972, pages 456-458 Columbus, Ohio, U.S.A. H.J. SCHAEFFER et al.: "Enzyme Inhibitors. 25. An Equation to Calculate the Unknown K_i from Two Known Values of K_i in an R,S, and RS Series. Stereoselectivity of Inhibition of Adenosine Deaminase by (R)-, (S)-, and (RS)-9-(1-Hydroxy-2-alkyl and -aralkyl)adenines" * Pages 456-458 *	
D,A	JOURNAL OF MEDICINAL CHEMISTRY, vol. 60, no. 8, August 1971, pages 1204-1209 ./.	

CLASSIFICATION OF THE APPLICATION (Int. Cl. 3)

C 07 D 473/30
473/34
473/38
A 61 K 31/52//
C 07 D 473/40
239/48
C 07 C 97/02
91/04

TECHNICAL FIELDS SEARCHED (Int.Cl. 3)

C 07 D 473/00
A 61 K 31/52

CATEGORY OF CITED DOCUMENTS

X: particularly relevant
A: technological background
O: non-written disclosure
P: intermediate document
T: theory or principle underlying the invention
E: conflicting application
D: document cited in the application
L: citation for other reasons

&: member of the same patent family, corresponding document

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
The Hague	04-12-1979	NUYTS

EPO Form 1503.1 06.78

European Patent Office

EUROPEAN SEARCH REPORT

0009154

Application number

EP 79 10 3222

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim
	Washington D.C., U.S.A. H.J. SCHAEFFER et al.: "Enzyme Inhibitors XXIV: Bridging Hydrophobic and Hydrophilic Regions on Adenosine Deaminase" * Pages 1204-1209 * ----	

CLASSIFICATION OF THE APPLICATION (Int. Cl. 3)

TECHNICAL FIELDS SEARCHED (Int. Cl. 3)